Exhibit I

Malignant Mesothelioma Arising after Direct Application of Asbestos and Fiber Glass to the Pericardium[1, 2]

ANDREW CHURG, MARTHA L. WARNOCK, and KLAUS G. BENSCH

SUMMARY

A case of mesothelioma, apparently arising in the pericardium, is reported in a patient who, 15 years previously, had been treated for angina pectoris by dusting of the pericardial cavity with a mixture of fibrous dusts. At autopsy, transparent fibers and ferruginous bodies were present within the pericardium. Electron diffraction and microprobe analysis indicated that approximately two thirds of the fibers were tremolite and anthophyllite asbestos, and the remainder, fiber glass. Development of mesothelioma in laboratory animals has been reported after intrapleural deposition of asbestos and other fibers, but in humans, the link between exposure to asbestos and mesothelioma has always been based on epidemiologic data and the retrospective finding of asbestos in tissues. To our knowledge, this is the first example of a malignant mesothelioma in a human associated with direct mesothelial contact with fibrous dusts.

Introduction

The epidemiologic association of exposure to asbestos and the subsequent development of pleural and peritoneal mesotheliomas is well established. A history of exposure to asbestos can be elicited in a large percentage of patients presenting with this tumor; typically, the initial or only contact with the dust is 15 to 50 years before the appearance of the neoplasm (1–3). The carcinogenic properties of other inhalable fibrous dusts are uncertain; however, it has been shown that intrapleural instillation of a variety of mineral fibers, including asbestos and fibrous glass, will all produce mesotheliomas in laboratory animals (4–7). We report a patient with a malignant mesothelioma arising after direct exposure of mesothelial surfaces to a mixture of asbestos and fiber glass.

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Case Report

A 61-year-old man was first seen at the Stanford University Medical Center in February 1972 with a 3-month history of progressive dyspnea. He had been in good health until 1954, when he developed angina pectoris; this was followed by a myocardial infarction in 1956. Thereafter, he experienced intractable angina, and in 1957 he underwent pericardiopexy in an attempt to improve collateral coronary circulation. The surgical procedure consisted of opening the pericardial sac, scarifying the epicardium, and dusting 0.3 g of asbestos over the epicardial surface. The pericardial sac was not closed, but mediastinal fat was sutured over the defect. Postoperatively, the patient improved dramatically, with complete disappearance of angina and normal exercise tolerance. He resumed work as an engineer in a paint factory where he was exposed to lead dust. The patient always wore a mask during exposure periods, and repeated blood lead concentrations were within normal limits. The patient never smoked.

The patient remained well until the onset of dyspnea and loss of appetite in late 1971. At that time, physical examination revealed dullness over the right chest, and a chest radiograph showed a large right pleural effusion. One year previously, the chest film had been normal. There was no evidence of heart

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failure. Examination of the pleural fluid showed many malignant cells. After thoracentesis, the chest film revealed that the heart was slightly enlarged, and that a 2-cm pleural mass was present in the first right intercostal space. The pleura itself did not appear to be thickened. At thoracotomy, multiple small tumor nodules were seen studding the visceral and parietal pleura, as well as the diaphragm; biopsy of the nodules were interpreted as malignant mesothelioma.

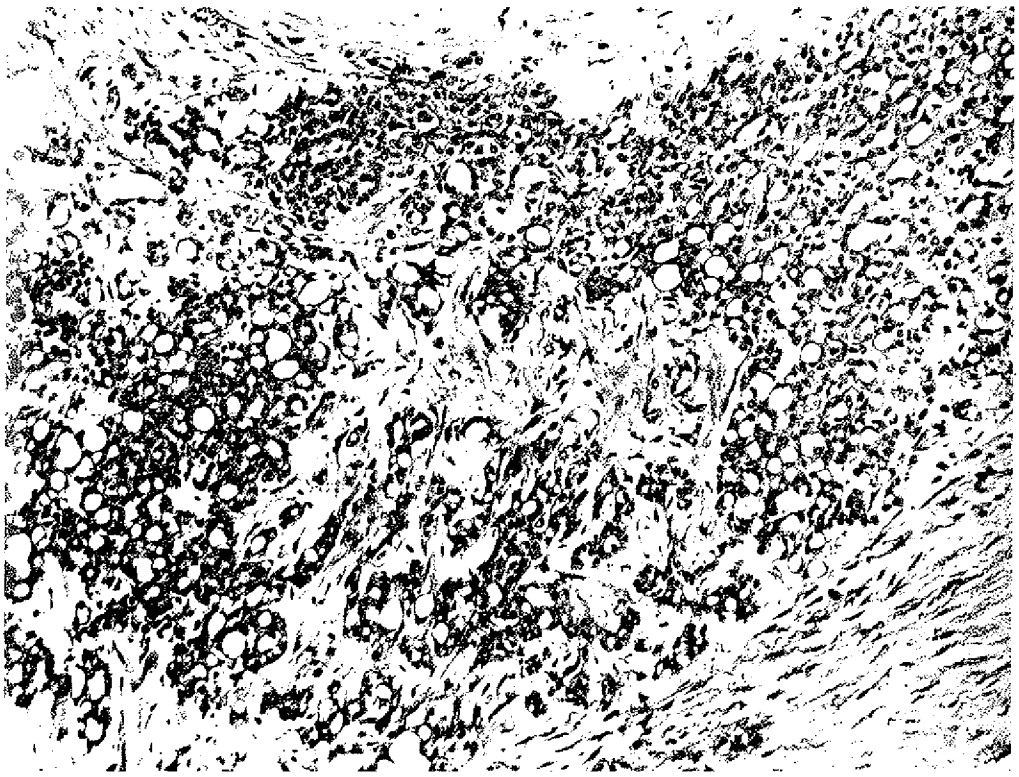
No treatment was given until June 1972, when the patient developed a tumor mass at the thoracotomy site. The tumor was treated with 3,000 rads and disappeared; Quinicrine was injected intrapleurally to control recurrent effusions. By January 1973, he experienced severe right-sided chest pain, fatigue, and shortness of breath. Treatment with cyclophosphamide, 5-fluorouracil, and Vincristine failed to effect an improvement. The patient's condition progressed, and he died in October 1973. An autopsy was performed.

Case Report

Tissue for light microscopy was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin, Perl's stain for iron, mucicarmine, periodic acid-Schiff stain with and without diastase, and colloidal iron with and without hyaluronidase. Tissue from the biopsy was fixed for electron microscopy in glutaraldehyde; additional tissues for electron microscopy consisted of autopsy material initially fixed in formalin, and tissues in paraffin blocks. All tissues for electron microscopy were postfixed in osmium tetroxide, dehydrated in ethanol, and embedded in Maraglas.® Electron diffraction and electron microprobe analysis were performed on thin sections and were interpreted as described previously (8, 9). Fiber sizes, as seen in the light microscope, were calculated using a calibrated eyepiece micrometer.

Gross findings. At autopsy, numerous subcutaneous masses were noted over the right chest wall. Bilateral sanguinous effusions were present within the chest, and the right parietal pleura was studded by tumor nodules ranging from 0.5 to 4 cm in diameter. The heart, basal parts of the right middle and lower lobes of the lung, and right diaphragm were bound together by neoplastic tissue forming a large tumor mass (figure 1). A part of this mass obliterated the pericardial sac in the region of the right atrium and ventricle (dense fibrous adhesions obliterated the remainder of the pericardial sac) and extended into the right pleural space, where it formed the sheet of tumor tissue that surrounded the basal parts of right lower and middle

Fig. 1. The center of the tumor mass, present at the junction between pericardium and right pleura, formed a broad mass of tissue occupying the right half of the pericardial sac and basal parts of the right pleural space, particularly between diaphragm and lung. Tumor is seen to have invaded the right atrial wall (*short arrow*) and penetrated through the diaphragm (*long arrow*).



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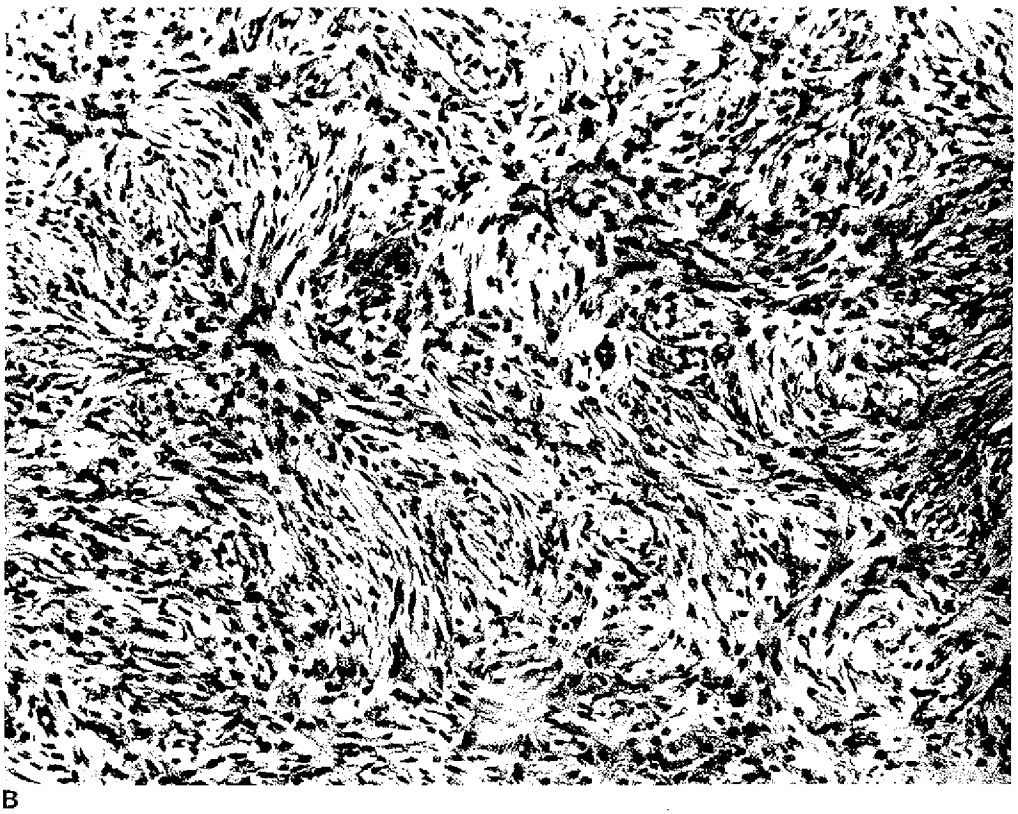

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Fig. 2. A. Area of tumor showing pattern of small glands lined by flattened cells (hematoxylin and eosin stain; original magnification: × 250). B. Area composed of spindled cells and occasional giant cells. These appearances are typical of mesothelioma (hematoxylin and eosin stain; original magnification: × 350).

lobes of the lung. The tumor also extended into and, at several points, through the right diaphragm. Close inspection showed narrow bands of tumor invading the myocardium and subpleural parts of the lung parenchyma. A 6-cm tumor mass was present on the undersurface of the right diaphragm, compressing, but not invading, the liver. No separate foci of tumor were present within the right lung, but 3 small metastases were found in the left lower lobe. Three periaortic nodes also contained metastatic tumor.

Microscopic findings. Sections of the surgical pleural biopsy revealed a tumor with a biphasic pattern. One pattern was composed of small glands of circular configuration lined by flattened or cuboidal cells (figure 2A). The nuclei were generally oval or indented with small nucleoli and finely stippled chromatin. Mitoses were infrequent. The glands were usually packed in a back-to-back arrangement, and the lumina contained a faintly basophilic secretory product that stained with colloidal iron and was removed by hyaluronidase. Mucin stains were negative. The same cells formed small papillary masses where they occurred on free surfaces. The second pattern was composed of spindled cells with nuclear features similar to those in the glandular areas; the spindled cells formed fascicles and sometimes a storiform pattern (figure 2B). Rare giant cells were present. The 2 histologic patterns were generally not intermixed, although occasional small glands were present in the spindled areas. A diagnosis of malignant mesothelioma was made on the biopsy specimen. At autopsy, the primary and metastatic tumors were histologically similar.

Over the heart, the tumor cells were embedded in an enormously thickened and densely fibrotic pericardium. Even where no tumor was present, the fibrous reaction had fused the heart and lung. Embedded in this thickened pericardium were transparent refractile fibers and rare ferruginous bodies (figure 3). Fibers were not obvious within the tumor nodules themselves, and were not seen in the pleura. Iron stains failed to reveal ferruginous bodies in the pleura, lung, or spleen. Fibers were not found in the myocardium, ex-

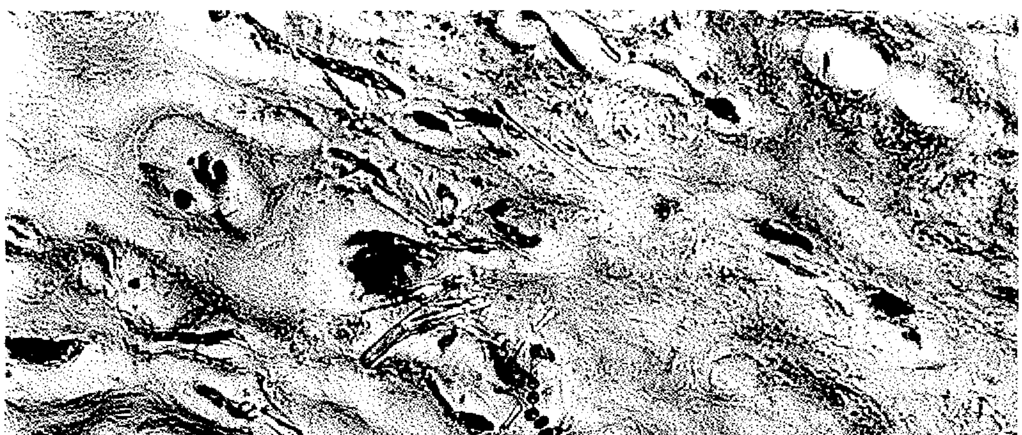

Fig. 3. Photomicrograph of fibrotic pericardium showing a ferruginous body (*arrow*) and numerous refractile uncoated fibers. Electron diffraction and microprobe analysis indicated that the fibers are amphiboles and fiber glass (hematoxylin and eosin stain; original magnification × 550).

TABLE 1

ELEMENTAL COMPOSITIONS OF FIBERS EXAMINED BY ELECTRON MICROPROBE ANALYSIS

Element	Anthophyllite (2 fibers)	Tremolite (9 fibers)	Fiber glass (4 fibers)
Mg	29.9	19.5	5.2
Al	9.1	8.5	13.6
Si	57.0	54.5	74.6
Ca	0.5	13.6	6.4
Fe	3.7	4.0	0.5

Values are given as per cent of each element and represent the average for all fibers of a given type that were examined.

cept at the junction with the epicardium. By light microscopy, the uncoated fibers in the pericardium were 2 to 200 μm in length; most were less than 50 μm long. Widths ranged from less than 1 to 5 μm; 75 per cent of the fibers were less than 1 μm wide.

Electron diffraction was performed on 50 sequentially counted fibers. Of these, 33 produced a diffraction pattern consistent with an amphibole asbestos, and 17 were amorphous. The width range for the amphiboles was 0.09 to 1.6 μm (average, 0.50 μm); for the amorphous fibers, the range was 0.16 to 1.6 μm (average, 0.69 μm). Electron microprobe analysis was performed on 15 fibers. The results are shown in table 1. Of the 11 fibers that produced amphibole diffraction patterns, 2 were chemically consistent with anthophyllite asbestos; the remaining 9, with tremolite asbestos. The 4 amorphous fibers examined consisted largely of aluminum, silicon, and calcium, and appeared to be fiber glass.

Discussion

The mechanism of asbestos-induced mesothelioma in the pleura or peritoneum is not entirely clear. It is believed that in the lung the fibers penetrate the pleura and exert their effects locally; counts of asbestos fibers in the lungs of patients with mesothelioma and no known asbestos exposure have been higher than in control subjects (10), but it is obviously difficult to devise a method for counting fibers present in the pleural space. Experimental instillation of asbestos fibers into the pleural cavity of rats induces mesothelioma with a high frequency. This frequency varies with the type of fiber present (amphiboles are more potent carcinogens than chrysotile) and is not an effect of oils, which may stick to the fibers during milling (4). Other nonasbestos fibers, such as fibrous glass, ceramic fibers, and brucite also may induce mesothelioma (4–7). Careful examination of the data suggests that the effect is a physical one that depends critically on fiber length and diameter; fibers of length greater than 8 μm and width less than 1.5 μm are especially oncogenic (6, 7).

The original surgeon's notes state explicitly that in this and other cases, "asbestos" fibers were dusted over the pericardial surface after mechanical scarification. We have been unable to determine the source of this "asbestos," but the diffraction and microprobe data clearly indicate that it consists of a mixture of amphiboles and fibrous glass. The ability of amphibole asbestos to form asbestos bodies is well documented (8, 9, 11, 12). Unfortunately, we were unable to examine the bodies present in this patient's tissue to determine whether any might have been formed on the fibrous glass. The particular varieties of asbestos used in this operation are somewhat unusual, because tremolite and anthophyllite are not generally used in commercial asbestos products. Exact identification of the mineral species present is probably less important than the fact that the size range, especially the diameters, of the fibers fits into the most carcinogenic range as detected in animal experiments (7). It has been suggested that fiber size, rather than fiber composition, is the most important determinant of fiber carcinogenicity (6, 7).

We have chosen to label the tumor in our patient as a pericardial mesothelioma, a type not usually associated with exposure to asbestos (1). This patient's clinical presentation is somewhat unusual for pericardial mesothelioma, because that tumor usually mimics an exudative pericarditis with compression of the cardiac chambers. Typically, the heart is greatly enlarged in radiographs and at autopsy presents a globular appearance with the tumor largely confined

within the greatly distended pericardial sac (13). In the present instance, the pericardium had already been opened surgically, so that a primary tumor in that site might easily have grown out and along the lung and pleura. Furthermore, obliteration of the pericardial space over the left ventricle and atrium by connective tissue, presumably laid down shortly after the scarification procedure, must have modified the usual pattern of growth of a neoplasm in this site. It is of interest to note that a greater fibrogenic response to dusts has been associated with a greater risk of mesothelioma in laboratory animal models (7).

The histologic appearance of the present tumor was typical of the mixed pattern of mesothelioma. It has been suggested that this pattern is the one most frequently associated with exposure to asbestos (14).

Whether the tumor is of pericardial or pleural origin, the present case represents, to our knowledge, the first example of malignant mesothelioma in a human arising after direct contact between a mesothelial surface and fibrous dust and, as such, provides support for the epidemiologic and experimental evidence cited.

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Exhibit J

Industrial Health 2005, **43**, 346–350

Case Report

An Autopsy Case of Primary Pericardial Mesothelioma in Arc Cutter Exposed to Asbestos through Talc Pencils

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Abstract: An autopsy case of a primary pericardial mesothelioma in a 53-year-old arc cutter is reported. He had often had the chance to inhale dust generated by sharpening the slate pencils composed of talc. He was admitted for heart failure due to pericardial tumor, but later died. The tumor was mainly located on the pericardium with a thickness of about 2.5 cm. Small nodular disseminations were observed in the left parietal pleura. Microscopically, tumor cells were epithelial-like and rich in histochemical demonstrable hyaluronic acid. Findings of immunohistochemical markers revealed keratin (+), EMA (+), calretinin (+), and CEA (–), which were characteristics of mesothelioma of epithelial type. The number of asbestos bodies (AB) in the lung parenchyma was increased (2,026 AB/gram dry lung tissue). Subsequent transmission electron microscopic examination equipped with an energy dispersive X-ray analyzer revealed that the fibers identified in the lungs were fibrous talc and actinolite. These findings suggested that this patient had been occupationally exposed to asbestos contaminated in the talc pencils, which induced the development of primary pericardial mesothelioma.

Key words: Pericardial mesothelioma, Asbestos, Talc pencil, Fiber analysis

Primary pericardial mesothelioma is an extremely rare and lethal cardiac tumor[1)]. A correlation has been established between exposure to asbestos and the development of pleural and peritoneal mesothelioma in many epidemiological surveys[2)]. However, the etiology of pericardial mesothelioma has not been elucidated. We report a case of pericardial mesothelioma occurring in an arc cutter who had used talc pencils, and examined the association between occupational exposure to asbestos and the development of this tumor by mineral analysis.

A 53-years-old man was admitted to our hospital because of shortness of breath and leg edema. He had been working for an ironworks for 28 yr. He had used slate pencils composed of talc when he drew the lines of arc cutting on iron plates since he was 26 years-old. He had often inhaled the dust generated when sharpening the talc pencils using sandpaper without respirator. He had smoked 2 packs of cigarettes for 18 yr.

Physical examination revealed distended jugular veins and leg edema. Breath sounds were decreased throughout the entire left lung field, but no fine crackle and no heart murmur was audible. His liver edge was palpable five fingerbreadths below the right costal margin.

Chest radiography on admission revealed cardiac enlargement and bilateral pleural effusion (Fig. 1a). Computed tomographic scan of the chest revealed bilateral

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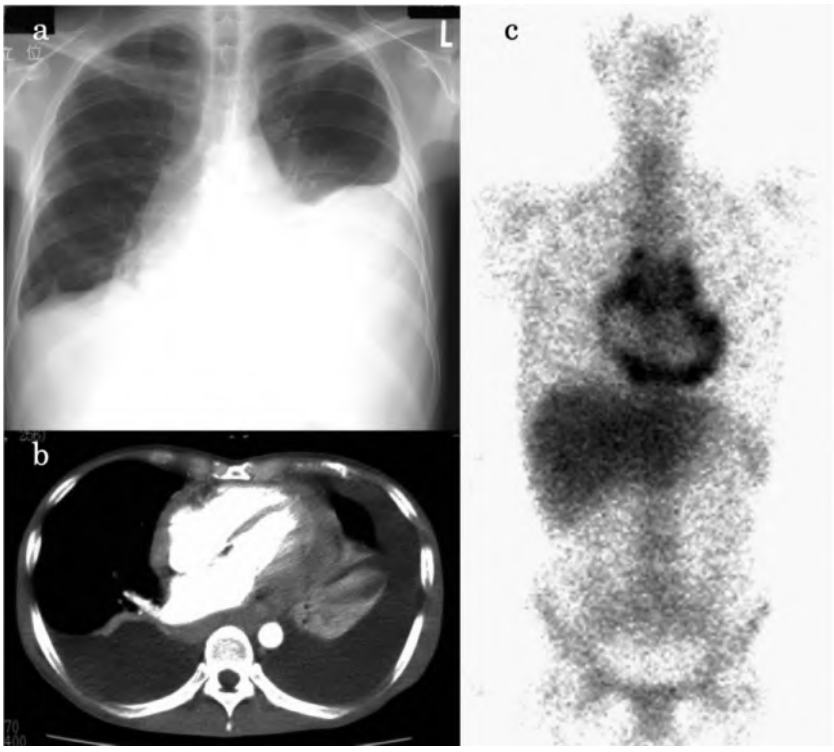

Fig. 1. (a) Chest radiograph taken on admission, showing cardiomegaly and bilateral pleural effusion. (b) Computed tomographic scan of the chest showing neoplastic thickening of the entire pericardium and bilateral pleural effusion. The left ventricle was compressed by tumor. (c) Galium-67 scintigraphy revealing an abnormal accumulation of radioisotope in the pericardial tumor.

pleural effusions, a neoplastic thickening that occupied the entire pericardial space without pericardial effusion and compressed the left ventricle (Fig. 1b). However, it did not reveal the findings of pleural plaque and pneumoconiosis. Gallium-67 scintigraphy revealed abnormal accumulation of radioisotope in the cardiac tumor (Fig. 1c). We performed several examinations in order to search for another primary lesion, but no lesion except the pericardial tumor was recognized.

Laboratory findings revealed an increased white blood cell count (12,600 /mm^3), elevations of serum bilirubin (2.3 mg/dl) and C-reactive protein (10.5 mg/dl), and normal levels of tumor markers such as CEA, CA19-9, and α-fetoprotein. Thoracocentesis yielded a clearly serous effusion, but its level of hyaluronic acid (18,600 ng/ml) was not so high as typical mesothelioma. Cytological examination of this aspiration revealed atypical cells with large eosinophilic nuclei.

The patient was clinically diagnosed with primary pericardial mesothelioma based on these imaging and cytological findings. As a result of communication with the patient and his family, we decided to do palliative treatments to relieve his symptoms instead of the chemotherapy. He died four months from the first admission, and consent for an autopsy was obtained from his family. The primary tumor was mainly located over the entire pericardium with a thickness of about 2.5 cm (Fig. 2). It extended to the cardiac base and compressed the superior vena cava and cardiac chambers. Microscopically, depositions of iron particles and mild pulmonary fibrosis were found. No pleural plaque was detected, but small nodular lesions, which were diagnosed as disseminations of the pericardial tumor, were observed in the left parietal pleura. The tumor cells were cuboidal cells with rounded nuclei, forming tubular and papillary structures (Fig. 3). Histochemical examinations demonstrated the presence of periodic acid-Schiff-positive and hyaluronidase-digestible components, indicating that cells were rich in histochemically demonstrable hyaluronic acid. Evaluation of immunohistochemical markers revealed a positive reaction to keratin, EMA, and calretinin (Fig. 3), and a negative reaction to CEA, which were characteristics of mesothelioma

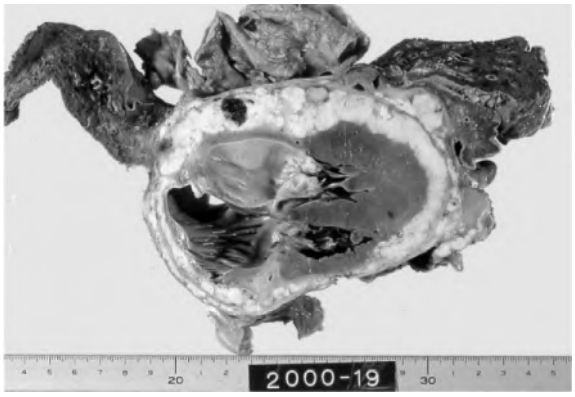

Fig. 2. Gross view at autopsy revealed that the heart was entirely encased by yellowish tumor, which occupied the pericardial space.
Note that the ventricular spaces are compressed by the tumor.

of epithelial type.

We determined the amounts of asbestos bodies (AB) by the filtration method after digesting the pericardial tumor and lung tissue[3]. There was no asbestos body in the primary tumor, but mildly increased number of asbestos bodies (2,026 AB/gram dry tissue) was observed in lung parenchyma. Analytical transmission electron microscope equipped with an energy dispersive X-ray analyzer (ATEM) revealed a large number of iron particles due to exposure to metal fumes, several fibrous talc and asbestos fibers in the lung parenchyma (Fig. 4). The energy dispersive X-ray (EDX) spectrum of the fibers showed typical characteristics of the talc and actinolite fiber (asbestos) (Fig. 5). No chrysotile fibers were detected by this ATEM analysis on many specimen fields although it was a quantitative analysis.

We reported an autopsy case of a worker who had worked as an arc cutter and died of primary pericardial mesothelioma, and the mineral analysis of his lung showed that he was occupationally exposed to asbestos.

Primary pericardial tumor is extremely rare, reported incidence of less than 0.0022% among 500,000 with autopsy cases[4]. Of such tumors, pericardial mesothelioma is probably the most common type. Hillerdal reviewed 4,710 cases of mesothelioma, and found that 88.8% of the cases were of pleural origin, 9.6% were peritoneal, 0.6% were both, 0.7% were pericardial, and 0.2% were in the tunica vaginalis testis[5]. Murai reviewed 1,846 cases of mesothelioma in Japan, and reported that the percentage of cases of pericardial mesothelioma was 6% (108 cases)[6]. Thomason et al reviewed 28 cases of pericardial mesothelioma described in the English literature from 1972 through 1992[1], and reported a male: female ratio of 2:1 and wide range of age

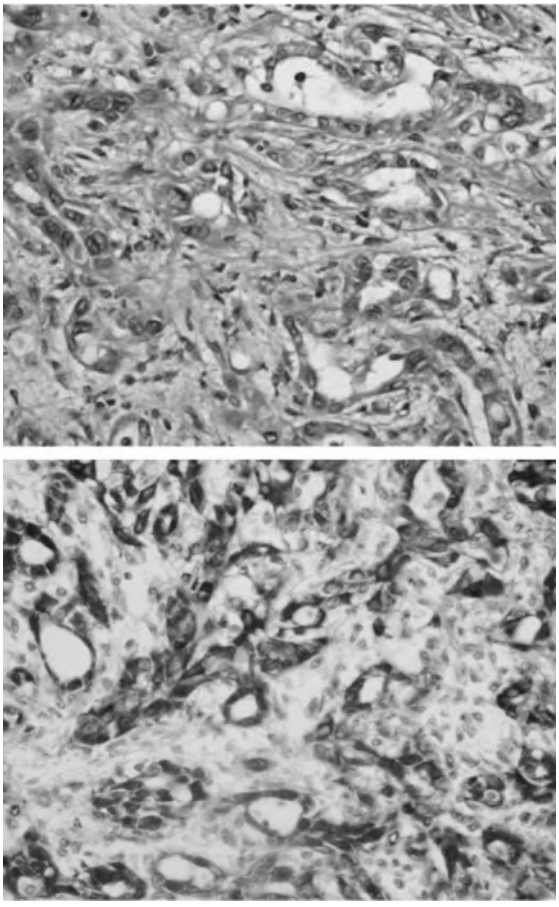

Fig. 3. (a) High-power photomicrograph of the tumor tissue demonstrates tubulo-papillary and grandular structure of cuboidal cells with rounded nuclei (original magnification, × 600, Hematoxylin-Eosin stain). (b) The tumor cells are positively stained immunohistochemically for anti-calretinin antibody (original magnification, × 600).

(12–77 yr; mean 47 yr).

The symptoms of the pericardial mesothelioma are nonspecific, such as chest pain, dyspnea, cough, and edema, so that most patients are diagnosed at an advanced stage. Computed tomography of the chest, magnetic resonance imaging, gallium-67 scintigraphy, and echocardiography can be of great help in making the diagnosis. However, precise diagnosis requires surgical biopsy including histological and immunohistochemical examinations. At present, there is no specific therapy for this disease. The median survival from the time of diagnosis is less than 6 months[7].

Ozer reported that pleural mesothelioma often invades to the pericardial space in the advanced phase of disease[8]. Therefore, our case must be distinguished from pericardial invasion by pleural mesothelioma. In our case, the tumor was mainly located in the pericardial space, and there was

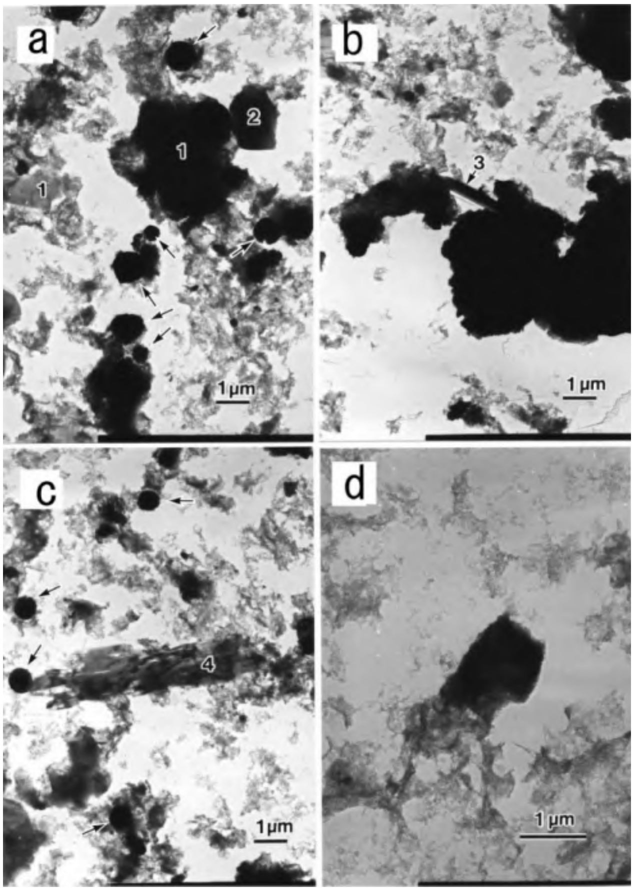

Fig. 4. Representative particles and fibers found in the lung parenchyma (a) (b) (c) and pericardial tumor (d) evaluated by transmission electron microscopy.
Black arrows: iron particles (metal fumes). No. 1: chlorite. No. 2: quartz. No. 3: fibrous talc. No. 4: actinolite asbestos. Particle in (d): talc coated by iron-protein (talc body).

no continuity between the pericardial tumor and small lesions scattered in the left pleural space. These findings support that the pericardial lesion was the primary.

The epidemiological association of exposure to asbestos and subsequent development of pleural and peritoneal mesothelioma is well-established[2]. Unlike pleural or peritoneal mesothelioma, no definite association between previous exposure to asbestos and the development of this tumor has been established. Thomason et al. reported that exposure to asbestos had been documented in four of 28 cases (14%)[1]. Beck *et al.* reported three cases of primary pericardial mesothelioma associated with occupational asbestos exposure[9]. In Japan, Kishimoto, *et al.* reported a female case of pericardial mesothelioma who had been working in Navy dockyard, and found asbestos bodies in her lung[10].

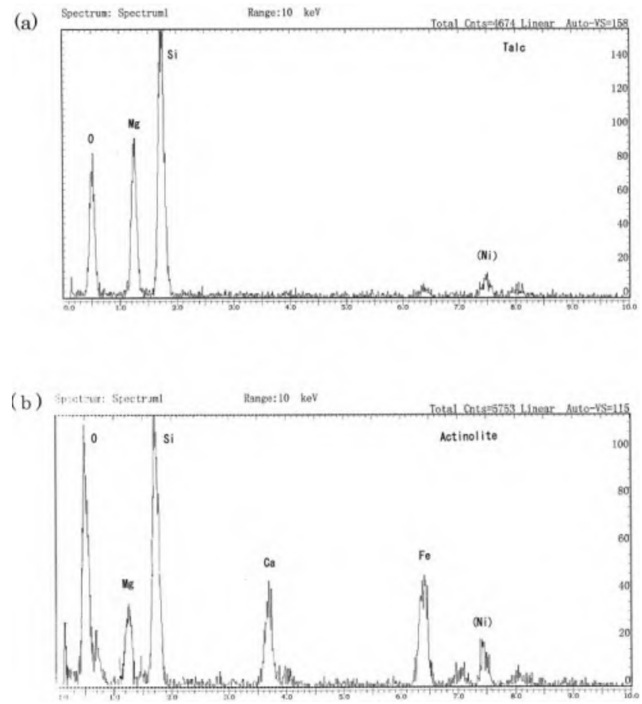

Fig. 5. The energy dispersive X-ray (EDX) spectrum of the fibers identified in the lung parenchyma by transmission electron microscopy.
(a) EDX spectrum of the fiber No.3 indicates the characteristics of the fibrous talc. (b) EDX spectrum of the fiber No.4 indicates the characteristics of actinolite asbestos.

Our patient had worked as an arc cutter for 28 yr and had drawn cutting lines on metal plates using slate pencils. He had inhaled dust generated from sharpening of this pencils. Slate pencils are manufactured from natural rock mainly consisting of talc, which is well known to cause ‘talc’ pneumoconiosis[11]. Talc is also used in numerous manufacturing industries for use in paints, ceramics, plastics, rubber products, paper, cosmetics, and pharmaceuticals[12]. Talc contains various percentages of asbestos fibers as an impurity[13], so that workers handling talc might be at risk of asbestos exposure. Although welders are well known to be at high risk for asbestos exposure[14], few studies have mentioned about a risk for asbestos exposure in arc cutters. In this case, there was no morphologic change of exposure to asbestos such as pleural plaques, but we found increased amounts of asbestos bodies in lung parenchyma (2,026 AB/gram dry tissue) above the minimum level for occupational asbestos exposure proposed by Kohyama *et al.*[15]. Furthermore, ATEM analysis revealed that fibers detected in the lung parenchyma were actinolite, and no other asbestos fibers were found by the qualitative analysis. Actinolite is

also known to be carcinogenic to animals as well as other types of asbestos[16, 17]. These findings suggest that this patient had been occupationally exposed to asbestos (actinolite asbestos), which might be highly relevant to the development of pericardial mesothelioma.

Asbestos-related diseases could be found in arc cutters handling talc pencils as well as arc welders. Some cases of primary pericardial mesothelioma might be associated with asbestos exposure. On September 19, 2003, primary malignant pericardial mesothelioma with occupational asbestos exposure was added in the list of prescribed diseases in Japan.

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Exhibit K

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National survey of malignant mesothelioma and asbestos exposure in Japan

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In the present study, malignant mesothelioma (MM) cases in Japan were investigated retrospectively. We extracted records for 6030 cases of death due to MM between 2003 and 2008 to clarify the clinical features of MM, including its association with asbestos exposure (AE). Of all these cases, a clinical diagnosis of MM was confirmed for 929. The origin of MM included the pleura in 794 cases (85.5%), the peritoneum in 123 cases (13.2%), the pericardium in seven cases (0.8%), and the testicular tunica vaginalis in five cases (0.5%). The histological subtypes of MM included 396 epithelioid (55.9%), 154 sarcomatoid (21.7%), 126 biphasic (17.8%), and 33 cases (4.7%) classified as ''other types''. Of all the MM cases, AE was indicated in 76.8% and pleural plaques were detected in 34.2%. The number of asbestos particles was determined in 103 cases of MM. More than 1000 asbestos particles per gram dried lung tissue were detected in 74.8% of cases and more than 5000 particles were detected in 43.7% of cases. We compared patient characteristics and the diagnostic procedures for MM before and after the ''Kubota shock''. Compared with the early phase of this study (2003–2005), the median age at diagnosis of MM was higher, the number of cases without definite diagnosis of MM was lower, the proportion of cases diagnosed by thoracoscopy was higher, and the percentage of cases in which the occupational history was described in the medical records was significantly higher in the later phase (2006–2008). Our study confirmed that more than 70% of MM cases in Japan are associated with AE. The ''Kubota shock'' may affect some features pertaining to MM. (*Cancer Sci* 2012; 103: 483–490)

Malignant mesothelioma (MM) is an aggressive tumor that develops from mesothelial cells of the pleura, peritoneum, pericardium, or testicular tunica vaginalis.[1] A newspaper article published in June 2005 reported that five residents who lived near the now-closed asbestos cement pipe plant in Amagasaki, Japan, developed pleural mesothelioma.[2] The asbestos-related problems that the article described caused considerable social concern, resulting in the so-called ''Kubota shock''. Asbestos has attracted increasing social attention, but no large-scale studies have been conducted to date investigating the clinical features of MM in Japan. In Japan, patients who have a history of occupational asbestos exposure (AE) and have developed MM are receive worker's compensation. However, of the 878 cases of death due to MM in 2003, only 85 cases were actually compensated (Ministry of Health, Labor and Welfare of Japan; http://www.mhlw.go.jp/houdou/2006/05/h0530-1.html, accessed 30 May 2006). Based on these figures, there is an urgent need to clarify the association between MM and AE in Japan, so we initiated the present retrospective survey to address this serious issue. We planned to investigate all MM cases in Japan and analyzed more than 6000 MM cases registered in the Vital Statistics yearly survey carried out by the Ministry of Health, Labour and Welfare. We have already reported the preliminary results of the analyses of the cases between 2003 and 2005.[3] In the present paper, we report the final results concerning the clinical features of MM in Japan between 2003 and 2008, with a particular focus on the association between MM and AE. The transition of some features of MM, such as patient characteristics and diagnostic procedures, before and after the Kubota shock is also discussed.

Materials and Methods

Study population. We requested and received authorization to view the death records in the Vital Statistics survey in Japan and extracted all the cases of death due to MM between 2003 and 2008. There were 6030 deaths due to MM (878 deaths in 2003, 953 in 2004, 911 in 2005, 1050 in 2006, 1068 in 2007, and 1170 in 2008), as shown in Figure 1. Based on the information in the death records, we contacted the closest living relatives by mail to obtain consent for our research. Consent was obtained in 2069 cases (34.3%). Based on authorization from the relatives, we applied to each medical institution to obtain medical information for the patients, including medical records, X-ray films, and/or computed tomography (CT) images. Information was obtained in 1111 cases (53.7%). The institutes and hospitals that provided information for our study are listed in Appendix I. We reviewed the medical records and radiological images to confirm a clinical and pathological diagnosis of MM. As a result, a clinical diagnosis of MM was confirmed in 929 cases (Fig. 2). The classification of pathological subtypes was based on World Health Organization criteria.[4] The clinical stage of MM was determined according to the criteria of the International Mesothelioma Interest Group (iMig).[5]

Assessment of AE. The patients' work histories, those of their family members, and residential histories for each case were investigated for AE. The residential histories of the patients covered the time period from their youth, which may suggest environmental exposure to asbestos based on information in their medical records. Questionnaires were also given to the patients' family members. Pleural plaques were assessed based on chest X-ray and/or CT images as the characteristic finding indicating AE.

Asbestos particle analysis. Asbestos particles were quantified based on lung tissues obtained from surgery or autopsy, which were provided from each medical institution, using the protocol modified by Kohyama and Suzuki.[6] Briefly, normal lung tissues (1–2 g) without tumor involvement were dehydrated at 100°C, weighed precisely, and then microcut. The tissues were digested in an aqueous solution containing 5–20% sodium

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EXHIBIT K

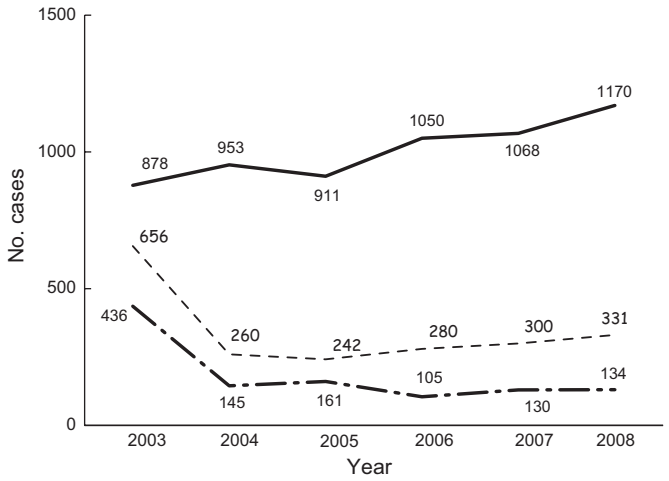

Fig. 1. Changes in the number of subjects over the study period. The solid line indicates the number of deaths due to mesothelioma. The dashed line indicates the number of cases in which consent was obtained from the closest living relative. The dashed-dotted line indicates the number of cases in which medical information was provided by the medical institution.

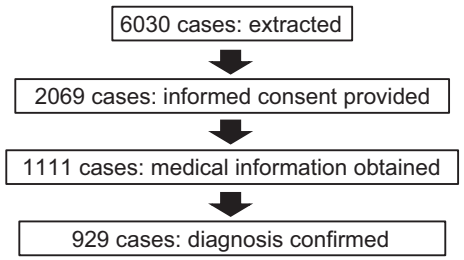

Fig. 2. Diagram showing case collection in the present study.

hypochlorite. Following digestion, samples were centrifuged at 1450g for 10 min and then resuspended in 50 mL distilled water. Samples were mixed well and suction filtered through a 0.45-μm Millipore filter (Merck Millipore, Tokyo, Japan). The filter was dehydrated and dried with acetone vapor. Asbestos particles were then counted using phase contrast microscopy and the number of asbestos particles per gram dried lung tissue was calculated. This analysis was performed at the Okayama Rosai Hospital.

Statistical analyses. Comparisons between independent groups were performed using the Chi-squared test and non-parametric analysis of the Mann–Whitney *U*-test. Mean values were compared using *t*-tests. Statistical calculations were performed with SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of patients with MM. Based on the description on the medical records and a review of the radiographs, a clinical diagnosis of MM was confirmed in 929 cases. These included 753 men (81.1%) and 176 women (18.9%). The median age at diagnosis was 67.0 years (range 16–94 years). The median age at the time of diagnosis of MM was significantly higher in the cases in which the patient had died between 2006 and 2008 (late phase) than in cases in which the patient had died between 2003 and 2005 (early phase; 69.4 *vs* 66.4 years, respectively; $P < 0.001$). In 101 cases (9.1%), a clinical diagnosis of MM was made on the basis of radiological or laboratory findings, such as hyaluronic acid in the pleural fluid, without pathological confirmation. We defined these cases as ''suspected MM''. The number of cases of suspected MM decreased in the late phase compared with the early phase (26 [7.0%] *vs* 70 cases [9.4%], respectively; $P < 0.001$). The origin of MM included the pleura in 794 cases (85.5%) of MM and 96 cases of suspected MM, the peritoneum in 123 cases (13.2%) of MM and five cases of suspected MM, the pericardium in seven cases (0.8%) of MM, and the testicular tunica vaginalis in five cases (0.5%) of MM. Eighty-one cases (7.3%) were excluded from further analysis because they were diagnosed as having other diseases based on their clinical and pathological records. These included 33 cases diagnosed as lung cancer, four cases diagnoses as ovarian cancer, three cases diagnosed as solitary fibrous tumor, and three cases diagnosed as carcinomatous pleuritis from unknown origin (Table 1).

Diagnosis of MM. The histological subtype of MM was indicated in the medical records in 709 cases (76.3%). Subtypes included 396 (55.9%) epithelioid cases, 154 (21.7%) sarcomatoid cases, 126 (17.8%) biphasic cases, and 33 (4.7%) cases from other types (Table 2). The diagnostic procedure was described in 891 cases and histological diagnosis was confirmed in 776 of 891 cases (87.1%). Pathological specimens were obtained by thoracoscopic biopsy (46.1%), transcutaneous needle biopsy (32.4%), thoracotomy (18.4%), autopsy (1.8%), or other procedures in MM cases of the pleura. In cases of MM of the peritoneum, specimens were obtained by laparotomy (62.7%), laparoscopic biopsy (23.5%), transcutaneous needle biopsy (7.8%), or other procedures. Diagnoses were made on the basis of cytological analysis of the pleural fluid in 94 MM cases of the pleura, the ascites in 19 cases of MM of the peritoneum, and the pericardial fluid in two cases of MM of the pericardium.

We also investigated changes in diagnostic procedures. In the early phase, 150 cases (29.8%) were diagnosed by thoracotomy or laparotomy. This proportion decreased significantly decreased to 16.2% in the late phase ($P < 0.001$). Conversely, the proportion of cases diagnosed by thoracoscopy or laparoscopy

Table 1. Confirmed diagnoses

Diagnoses	No. cases (%)
Malignant mesothelioma	929 (83.6)
Pleura	794
Peritoneum	123
Pericardium	7
Testicular tunica vaginalis	5
Malignant mesothelioma (suspected)	101 (9.1)
Pleura	96
Peritoneum	5
Lung cancer	33 (3.0)
Lung cancer (suspected)	24 (2.2)
Ovarian cancer	4 (0.4)
Solitary fibrous tumor	3 (0.2)
Carcinomatous pleuritis	3 (0.2)
Others	14 (1.3)
Total	1111 (100)

Table 2. Histological subtypes of malignant mesothelioma

	Pleura (n = 606)	Peritoneum (n = 95)	Others (n = 8)	Total (n = 709)	%
Epithelioid	325	68	3	396	55.9
Sarcomatoid	141	11	2	154	21.7
Biphasic	111	12	3	126	17.8
Others	29	4	0	33	4.7

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Table 3. Detailed history of asbestos exposure in 824 mesothelioma cases

	No. cases (%)
Occupational exposure	607 (73.7)
Neighborhood exposure	18 (2.2)
Home exposure	8 (0.9)
Total	633 (76.8)

increased significantly from 34.0% in the early phase to 58.8% in the late phase ($P < 0.001$). Immunohistochemical analysis was performed in 734 (97.0%) of cases diagnosed on the basis of histological findings, as well as in 55.4% of cases diagnosed on the basis of cytological analysis of the pleural fluid, ascites, or pericardial fluid. Immunohistochemical analysis was performed in 39.7% of cases diagnosed using body fluids in the early phase, and this percentage increased significantly to 82.4% in the late phase ($P < 0.001$).

History of AE. A history of AE was investigated in 929 cases that had a definite diagnosis of MM. Information regarding AE was obtained through the medical records and/or questionnaires administered to the patients' families in 824 cases (88.7%). Among these, occupational AE was indicated in 607 cases (73.7%; Table 3). In 18 cases, AE was the result of an asbestos source in the neighborhood; nine of these cases had a history of residing in Amagasaki city, around the Kubota plant. In eight cases, AE in the home was indicated (i.e. the patient's husband or another family member had a history of occupational AE). As a whole, AE was indicated in 633 cases (76.8%). Of the 584 patients who died between 2004 and 2008 in whom occupational AE was indicated, an occupational history was given in the medical record for 205 cases (47.7%). The questionnaire administered to family members revealed occupational AE in 363 cases (84.4%).

Occupational category of AE. Table 4 divides the 607 cases with a history of occupational AE into different occupational categories. In cases where the patient had multiple jobs that involved AE, the job with the longest work history was selected. On the basis of this analysis, 131 patients (21.6%) had worked in the construction industry, 91 patients (15.0%) had worked in the shipbuilding industry, 46 patients (7.6%) had worked in plumbing, 44 patients (7.2%) had performed electrical work, 41 patients (6.8%) had worked in steel mill production, 36 patients (5.9%) had worked in manufacturing asbestos products, 35 patients (5.8%) had worked in automobile manufacturing, and 33 patients (5.4%) had worked in demolition. The occupational history was provided in the medical records for 28.7% of patients in the early phase, with this percentage increasing significantly to 62.4% in the late phase.

Table 5. Duration of asbestos exposure and latency of mesothelioma

	Median	Range
Mesothelioma of the pleura (*n* = 540) (years)		
Duration	30	0.25–58
Age at diagnosis	68	38–94
Latency	43.5	14–81
Mesothelioma of the peritoneum (*n* = 59) (years)		
Duration	28	1.5–54
Age at diagnosis	68	25–89
Latency	42.5	19–73
Total* (*n* = 607) (years)		
Duration	30	0.25–58
Age at diagnosis	68	25–94
Latency	43	13–81

*This includes five cases of pericardial mesothelioma and three cases of testicular tunica vaginalis mesothelioma.

Duration of AE. The duration of AE and the period of latency for the onset of MM were investigated in MM cases with occupational AE (Table 5). The median duration of AE was 43 years and the median period of latency for disease onset of MM was 43.7 years. In MM of the pleura and peritoneum, the median duration of AE was 30 and 28 years, respectively. The median period of latency for disease onset for MM of the pleura and peritoneum was 43.5 and 42.5 years, respectively.

Pleural plaques. The prevalence of pleural plaques was investigated based on chest X-ray or CT images provided by each medical institution. Pleural plaques were detected in 280 of 818 cases (34.2%), including 242 of 703 cases (34.4%) of MM of the pleura, 36 of 106 cases (33.9%) of MM of the peritoneum, one of seven cases (14.3%) of MM of the pericardium, and one of two cases (50%) of MM of the testicular tunica vaginalis. Based on these figures, we investigated the prevalence of pleural plaques in each job category with AE. For those who worked in the manufacture of asbestos products and shipbuilding, the

Table 4. Occupational category associated with asbestos exposure

Occupation	Pleura (*n* = 540)	Peritoneum (*n* = 59)	Pericardium (*n* = 5)	TTV (*n* = 3)	Total (*n* = 607)
Construction	120	9	1	1	131
Shipbuilding	81	8	1	1	91
Plumbing	43	2	1	0	46
Electrical work	40	4	0	0	44
Steel production	37	3	0	1	41
Asbestos products industry	25	11	0	0	36
Automobile manufacturing	32	3	0	0	35
Demolition work	31	2	0	0	33
Manufacturing	20	3	0	0	23
Chemical manufacturing	17	0	1	0	18
Warehouse management	13	4	0	0	17
Cement manufacturing	13	1	0	0	14
Shipman*	10	0	0	0	10
Transportation	10	0	0	0	10
Metal manufacturing	8	2	0	0	10
Others	33	7	1	0	41

*Shipman refers to someone who works on a boat in some capacity. TTV, testicular tunica vaginalis.

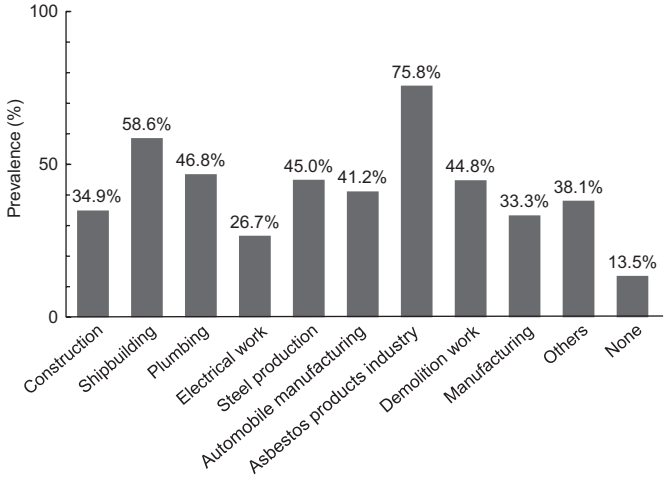

Fig. 3. Prevalence of pleural plaques, as determined by chest X-ray and/or computed tomography, for each occupational category.

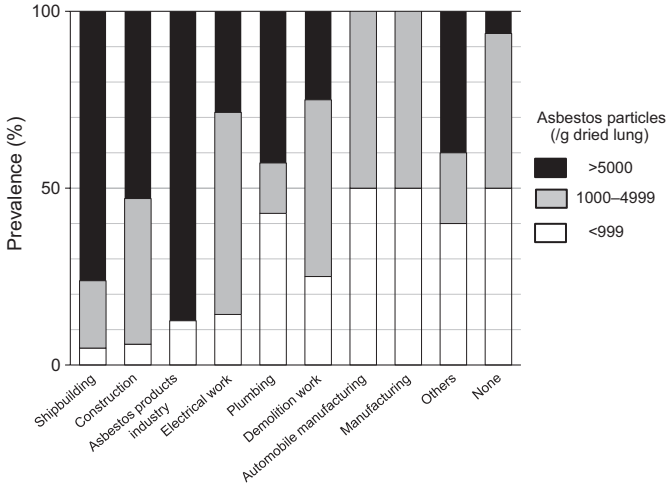

Fig. 5. Prevalence of asbestos particles in the lung of mesothelioma cases for each occupational category.

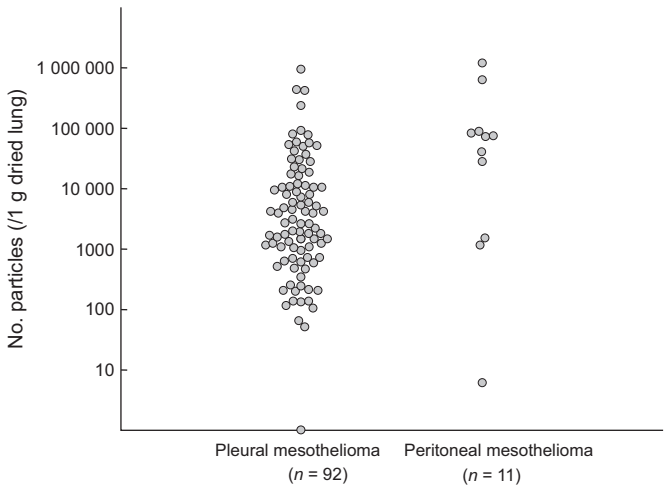

Fig. 4. Distribution of the number of asbestos particles in the lung for cases of pleural and peritoneal mesothelioma.

prevalence of pleural plaques was 75.8% and 58.6%, respectively (Fig. 3). Pleural plaques were also detected in 31 cases (13.5%) of MM cases without an obvious occupational history of AE, including two cases with a history of non-occupational AE.

Asbestos particles. The number of asbestos particles was determined in 103 MM cases (92 of the pleura and 11 of the peritoneum), in which lung tissue was provided by the medical institution. More than 1000 asbestos particles per gram dried lung tissue were detected in 77 cases (74.8%) and more than 5000 particles were detected in 45 cases (43.7%). Asbestos particles were more frequently detected in MM of the peritoneum than in MM of the pleura ($P = 0.046$), as shown in Figure 4. For patients with MM of the pleura, more than 1000 asbestos particles were detected in 67 cases (72.8%) and more than 5000 particles were detected in 37 cases (40.2%). For patients with MM of the peritoneum, more than 1000 particles were detected in 10 cases (90.9%) and more than 5000 particles were detected in eight cases (72.7%). The number of asbestos particles for each job category is shown in Figure 5. More than 5000 particles were detected in seven of eight patients (90.9%) who worked in the manufacture of asbestos products, in 16 of 21 patients (76.2%) who worked in shipbuilding, and in nine of 17 patients (52.9%) who worked in the construction industry. More than 1000 particles were detected in more than 90% of the patients who worked in the shipbuilding and construction industries. Asbestos particles were also detected in the cases without occupational AE. More than 5000 particles per gram dried lung were detected in one of 16 (6.3%) cases and more than 1000 particles were detected in eight of 16 (50%) cases. These included one case with a history of residence in Amagasaki city, near the Kubota plant (1042/g). We then analyzed the association between the prevalence of pleural plaques and asbestos particles in the lung and found an increased number of asbestos particles in cases of MM with pleural plaques ($P < 0.001$). It is of note that in more than half of the cases without pleural plaques (32/55; 58.2%) more than 1000 asbestos particles per gram dried lung were detected, with more than 5000 particles detected in 11 (25.0%) cases.

Discussion

In the present study, we investigated the features of 1111 MM cases for which medical information was provided by medical institutions following consent from the patient's closest living relative. We believe that the present study is the largest conducted in Japan into MM. There were 929 cases of confirmed MM and we regarded 101 cases (9.1%) as ''suspected MM'' because a pathological diagnosis had not been made. Compared with the early phase, the number of cases of suspected MM decreased significantly in the late phase. One possible explanation for this is that the propagation of immunohistochemical analysis may have contributed to definite diagnoses of MM. Immunohistochemical analysis was performed in most of the cases (97.0%) to obtain a diagnosis based on histological findings. An increased number of cases was diagnosed on the basis of cytological findings in the pleural fluid, ascites, or pericardial fluid in the late phase than in the early phase. Another possibility is the widespread dissemination of less-invasive diagnostic procedures. The cases of suspected MM include more elderly patients (median age 80 years) in the early phase and we suppose that these cases may have been excluded from diagnostic procedures. During the study period, the proportion of cases diagnosed using thoracotomy or laparotomy decreased significantly, whereas the number of cases diagnosed using thoracoscopy or laparoscopy increased significantly. Thoracoscopic exploration can be performed under a local anesthetic and an increased number of cases were diagnosed using this

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procedure.[7] Another possibility for the decrease in the number of cases of suspected MM is that patients with a pathologically confirmed diagnosis of MM could received Worker's Compensation or compensation from the Act on Asbestos Health Damage Relief established following the ''Kubota shock''. Dissemination of less-invasive diagnostic procedures, accurate pathological analysis techniques, and widening of coverage by the compensation system may have contributed to definitive diagnostic confirmation of MM in Japan.

A history of AE could be revealed in interviews with the patients and confirmed medically by the presence of pleural plaques and/or asbestos particles. In the present study, occupational AE was evident in more than 70% of MM cases. This percentage is consistent with that reported in other countries.[8] An association between MM and AE is well established worldwide.[9–11] Thus far, there have been two reports based on a small number of cases that suggested this association between AE and MM in Japan,[12,13] but no large-scale study has been conducted concerning the clinical features of MM in Japan. The median latency of disease onset is 43 years. Within the period of the present study, the percentage of cases in which the occupational history of the patients was described in their medical records increased significantly. These results indicate the widespread dissemination of knowledge to physicians concerning the association between MM and AE.

Asbestos particle analysis is one of the most reliable methods to evaluate AE. A level exceeding 1000 particles per gram dry lung tissue is associated with occupational AE and levels of more than 5000 particles are consistent with a doubling of the risk of lung cancer.[14] In the present study, these analyses revealed more than 1000 particles per gram dry lung tissue in 74% of cases examined. These results confirm that most of the MM cases in Japan are associated with AE. The number of asbestos particles was higher in cases in which the patients had an occupational history related to industries with a high level of AE, such as the asbestos products industry or shipbuilding. The friction materials used in automobiles or the asbestos plates used in manufacturing may contain chrysotile fibers, which are less likely to form asbestos particles than other types of asbestos. We think this may explain why automobile and manufacturing workers had comparatively low levels of asbestos particles in their lungs. Radiological examination detected pleural plaques in approximately 34% of cases (based on chest X-ray or CT). The prevalence of plaques was higher in patients whose occupation was in an industry in which the number of asbestos particles was at a high level of AE, such as the asbestos products industry or shipbuilding. Cases with pleural plaques were found to have significantly higher asbestos particle counts than those without plaques, even though more than half of the cases without plaques were found to have 1000 particles per gram dry lung tissue, including 25% of cases with more than 5000 particles per gram dry lung tissue. These findings suggest that the presence of pleural plaques is not sufficient for an evaluation of a history of AE. Physicians should be aware that even in cases without pleural plaques, occupational or environmental AE is not improbable.

In conclusion, our study confirms that more than 70% of cases of MM in Japan are associated with AE. The ''Kubota shock'' may affect some features pertaining to MM.

Acknowledgments

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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Appendix I: Medical institutions that provided medical information for the mesothelioma cases evaluated in the present study

Hokkaido. Family Clinic, Makita Hospital, Keiyukai Sapporo Hospital, Sapporo Minami Seisyu Hospital, Teine Keijinkai Hospital, Hokkaido Kin-Ikyo Chuo Hospital, Hakodate Hospital, Toya Onsen Hospital, Steel Memorial Muroran Hospital, Muroran City General Hospital, Hokkaido Prefecture Tomakomai Hospital, Sapporo City General Hospital, Sapporo-Kosei General Hospital, Sapporo Minami-Ichijo Hospital, Sapporo Minami-Sanjo Hospital, Hokkaido University Hospital, Hokkaido Social Insurance Hospital, KKR Sapporo Medical Center, NHO Hokkaido Medical Center, Hokkaido Ohno Hospital, Sapporo Higashi Tokushukai Hospital, Iwamizawa Hokusyokai Hospital, Asahikawa City Hospital, Fukagawa Municipal Hospital, Asahikawa-Kosei General Hospital, Asahikawa Medical University Hospital, Obihiro-Kosei General Hospital, Abashiri-Kosei General Hospital, Shimokawa Town Hospital.

Aomori. Aomori Municipal Hospital, Aomori Prefectural Central Hospital, Mutsu General Hospital, Hirosaki Chuo Hospital, Hirosaki University School of Medicine & Hospital, Aomori Jikeikai Hospital, Tsugaru Seijinbyo Center.

Akita. Akita Red-Cross Hospital, Yokote Municipal Hospital, Hiraka General Hospital, Senbokukumiai Hospital, Sasago Clinic, Koto General Hospital.

Iwate. Iwate Medical University Hospital, Iwate Prefectural Central Hospital, Iwate Prefectural Isawa Hospital, Tono Hospital, and Iwate Prefectural Takada Hospital.

Miyagi. Tohoku University Hospital, Sendai Kosei Hospital, Tohoku Rosai Hospital, Miyagi Cancer Center, Kurokawa Hospital, Tohoku Kosei Nenkin Hospital, NHO Sendai Medical Center, Saka General Hospital, Ishinomaki Red Cross Hospital, Tome City Hospital, Kesennuma City Hospital, Miyagi Cardiovascular & Respiratory Center, Furukawa Seiryo Hospital.

Niigata. Tachikawa Medical Center, Nagaoka Red Cross Hospital, Niigata Rosai Hospital, Niigata Prefectural Central Hospital, Keinan General Hospital, Kariwagun General Hospital, Niigata Prefectural Koide Hospital, Ojiya General Hospital, Tokamachi Hospital, Niigata Rinko Hospital, Kido Hospital, Niigata City General Hospital, Saiseikai Niigata Daini Hospital, Toyosaka Hospital, Niigata Cancer Center Hospital, Ryotsu Hospital, Sado General Hospital, Niitsu Medical Center Hospital, Kaetsu Hospital, Niigata Prefectural Shibata Hospital, Murakami Kinen Hospital, Suibaragou Hospital.

Fukushima. Iwase General Hospital, Tsuboi Cancer Center Hospital, Ohta Nishinouchi Hospital, Southern Tohoku Research Institute for Neuroscience Hospital, Takada Kosei Hospital, Kashima Hospital, Iwaki Kyouritsu Hospital.

Yamagata. Yamagata Saisei Hospital, Yamagata Prefectural Shinjo Hospital, Shonai Hospital, Saito Clinic, Tsuruoka Kyouritsu Hospital.

Ibaragi. Tsuchiura Kyodo General Hospital, JA Toride Medical Center, Tsukuba Medical Center Hospital, Johsai Hospital, The Prefecture West General Hospital, Ibaraki Prefectural Central Hospital, Hitachinaka General Hospital, Kashima Rosai Hospital, Hitachi General Hospital, NHO Ibarakihigashi National Hospital.

Tochigi. Tochigi Cancer Center, Dokkyo Medical University Hospital, Oyama Municipal Hospital, Ashikaga Hospital, Nogi Hospital, Hoshino Hospital.

Gunma. Komai Hospital, Maebashi Red Cross Hospital, Gunma University Hospital, Maebashi Kyouritsu Hospital, Saiseikai Maebashi Hospital, Isesaki Municipal Hospital, Gunma Prefectural Cancer Center, Tatebayashi Kosei Hospital, Fujioka General Hospital, Usui Hospital, Ohtashiyabuzukahonmachi Kokuho Clinic.

Saitama. Saitama Municipal Medical Center, Toda Chuo General Hospital, Saitama Red Cross Hospital, Maruyama Memorial General Hospital, Soka Municipal Hospital, Kuki General Hospital, Aida Hospital, Dokkyo Medical University Koshigaya Hospital, Higashisaitama General Hospital, Saiseikai Kurihashi Hospital, Saitama Medical University Hospital, Saitama Medical University Saitama Medical Center, Saitama Medical University International Medical Center, Higashimatsuyama Municipal Hospital, Musashi Ranzan Hospital, Japanese Red Cross Ogawa Hospital, Hanno Municipal Hospital, Toyooka-daiichi Hospital, Saitama Prefectural Cardiovascular & Respiratory Medical Center, Ageo Kousei Hospital, Saitama Cancer Center, Kitasato University Kitasato Institute Medical Center Hospital, Chichibu Seikyo Hospital, Minano Hospital.

Chiba. Inoue Memorial Hospital, Chiba Cancer Center, NHO Chiba-East-Hospital, Chiba Kaihin Municipal Hospital, Sakusabesaka Street Clinic, Kasori Hospital, Shinmatsudo Central General Hospital, Shirai Seijinkai Hospital, Nippon Medical School Chiba Hokusoh Hospital, Matsudo City Hospital, Aozora Clinic, Funabashi Futawa Hospital, Saiseikai Narashino Hospital, St. Marguerite Hospital, Kashiwa City Hospital, National Cancer Center Hospital East, Kikkoman General Hospital, Seirei Sakura Citizen Hospital, Toho University Medical Center Sakura Hospital, Sanmu Medical Center, Asahi Hospital, Chiba Rosai Hospital, Yarita Hospital, Kimitsu Chuo Hospital, Hananotani Clinic, Kameda Medical Center, Yamanouchi Hospital, Mobara Chuo Hospital, Teikyo University Chiba Medical Center, Satsukidai Hospital.

Tokyo. Sanraku Hospital, Tokyo Teishin Hospital, Tokyo Metropolitan Police Hospital, Jikei University Hospital, Saiseikai Central Hospital, Eiju Hospital, Tokyo Kensei Hospital, Nippon Medical School Hospital, The University of Tokyo Hospital, Juntendo University Hospital, Higashijujo Hospital, Ouji Hospital, Towa Hospital, Jieikai Hospital, Kameari Hospital, Homecare Clinic Kawagoe, Edogawa Hospital, Jukoukai Hospital, Juntendo Tokyo Koto Geriatric Medical Center, NTT Medical Center Tokyo, Yutaka Hospital, Showa University Hospital, Tokyo Rosai Hospital, Toho University Medical Center Omori Hospital, Kimura Hospital, Ikegami General Hospital, Naito Hospital, NHO Tokyo Medical Center, Toho University Medical Center Ohashi Hospital, Tokyo Kyosai Hospital, Nissan Tamagawa Hospital, Kanto Central Hospital, Keio University Hospital, Tokyo Denryoku Hospital, Tokyo Medical University Hospital, National Center for Global Health and Medicine, Tokyo Women's Medical University Hospital, Social Insurance Chuo General Hospital, Sekino Hospital, Toshima Hospital, Nihon University School of Medicine Itabashi Hospital, Tokiwadai Geka Hospital, Takashimadaira Clinic, Kyorin University Hospital, Nomura Hospital, Tokyo Metropolitan Tama Medical Center, NHO Disaster Medical Center, Tachikawa Sougo Hospital, Hinodegaoka Hospital, Tokai University Hachioji Hospital, Hachioji Sannou Hospital, Tokyo Medical University Hachioji Medical Center, Fukujuji Hospital, Tama-nambu Chiiki Hospital.

Kanagawa. St. Marianna University of Medicine Toyoko Hospital, Kanto Rosai Hospital, Inadanoborito Hospital, St. Marianna University of Medicine Hospital, Keiyu Hospital, Japanese Red Cross Tsukui Hospital, Kanagawaken Hospital, Yokohama Rosai Hospital, Showa University Northern Yokohama Hospital, Showa University Fujigaoka Hospital, Fuchinohe Hospital, Soai Hospital, Sagamihara Kyodo Hospital, Yokohama Central Hospital, Yokohama City University Medical Center, Byoubugaura Hospital, Yokohama City University Hospital, Yokohama Minami Kyousai Hospi-

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tal, Kanazawabunko Hospital, Kanagawa Cardiovascular and Respiratory Center, Yokosuka Kyosai Hospital, Yokosuka General Hospital Uwamachi, Seirei Yokohama Hospital, Yokosuka City Hospital, Yokohama City Hospital, St. Marianna University School of Medicine Yokohama Seibu Hospital, Kanagawa Cancer Center, Yamato Municipal Hospital, Ebina General Hospital, Syonan Izumi Hospital, Yokohama Sakae Kyosai Hospital, Kiyokawa Hospital, Shonan Memorial Hospital, Odawara Municipal Hospital, Shonan Central Hospital, Chigasaki Municipal Hospital, Chigasaki Tokusyukai General Hospital, Hiratsuka Kyosai Hospital, Ashigarakami Hospital, Isehara Kyodo Hospital, Tokai University Hospital.

Yamanashi. Kofu Kyoritsu Hospital, Yamanashi Kosei Hospital, University of Yamanashi Hospital.

Shizuoka. Numazu City Hospital, Seirei Numazu Hospital, Susono Red Cross Hospital, Juntendo University Shizuoka Hospital, Shizuoka Cancer Center, Hattori Clinic, Ito Hospital, Fuji City General Hospital, Fujinomiya Municipal Hospital, Shizuoka General Hospital, Haibara General Hospital, Kanbara General Hospital, Shizuoka Saiseikai General Hospital, Yamanoue Hospital, Yaizu City Hospital, Fujieda Municipal General Hospital, Shimada Municipal Hospital, Hamamatsu Rosai Hospital, Seirei Hamamatsu General Hospital, Hamana Hospital, Hamamatsu University School of Medicine University Hospital, Hamamatsu Medical Center, Seirei Mikatahara General Hospital, Hamamatsu Red Cross Hospital, Kakegawa Municipal General Hospital, Omaezaki Municipal Hospital.

Nagano. Aiwa Hospital, Saku Central Hospital, NHO Shinsyu Ueda Medical Center, Hokushin General Hospital, Karuizawa Municipal Hospital, Tomi Civilian Hospital, Aizawa Hospital, Suwa Red Cross Hospital, Iida Municipal Hospital.

Aichi. Toyokawa City Hospital, Gamagori City Hospital, Aichi Cancer Center Aichi Hospital, Nishio Municipal Hospital, Anjo Kosei Hospital, Hekinan Municipal Hospital, Kariya Toyota General Hospital, Meitetsu Hospital, Aichi Saiseikai Hospital, Japanese Red Cross Nagoya Daiichi Hospital, Nagoya Ekisaikai Hospital, Sumitomo Kinen Hospital, Chubu Rosai Hospital, Rinko Hospital, Kyoritsu General Hospital, Daido Hospital, Minami Seikyo Hospital, Nagoya Municipal Seibu Medical Center Johoku Hospital, Nagoya University Hospital, Japanese Red Cross Nagoya Daini Hospital, Nagoya City University Hospital, Nagoya Memorial Hospital, Aichi Kokusai Hospital, Fujita Health University Hospital, Toyota Memorial Hospital, Chubu Hospital, Handa City Hospital, Tokai Municipal Hospital, Chita City Hospital, Tokoname City Hospital, Aichi Medical University Hospital, Konan Kosei Hospital, Komaki City Hospital, Kasugai Municipal Hospital, Asahi Rosai Hospital, Tosei General Hospital, Daiyukai General Hospital, Aichi Cardiovascular & Respiratory Medical Center, Yamashita Hospital, Bisai Hospital.

Gifu. Gifu Municipal Hospital, Kishimoto Hospital, Ogaki Municipal Hospital, Kumiai Kosei Hospital, Gifu Social Insurance Hospital, Kosaka Kokuho Clinic, Tohno Kosei Hospital, Ena Municipal Hospital.

Toyama. Toyama University Hospital, Toyama Red Cross Hospital, Toyama Prefectural Central Hospital, Toyama Kyoritsu Hospital, Nanto Municipal Hospital, Toyama Rosai Hospital, Shinseikai Toyama Hospital, NHO Toyama Hospital, Miwa Hospital, Toyama City Hospital.

Ishikawa. Kanazawa Social Insurance Hospital, Kanazawa Medical University Hospital, Saiseikai Kanazawa Hospital, NHO Kanazawa Medical Center, Tsurugi Hospital, Eijukai Mirai Hospital, Komatsu Municipal Hospital, Noto General Hospital, Keiju General Hospital.

Fukui. Nitta Gastrointestinal and Radiology Hospital, Fukui General Hospital, Municipal Tsuruga Hospital, Fukui Red Cross Hospital, Fukui Saiseikai Hospital.

Shiga. Otsu Municipal Hospital, Social Insurance Shiga Hospital, Shiga University of Medical Science Hospital, Hikone Municipal Hospital, Shiga Medical Center for Adults, Kusatsu General Hospital, NHO Shiga Hospital.

Mie. Yokkaichi Municipal Hospital, Mie Prefectural Medical Center, Yamamoto General Hospital, Kuze Gastrointestinal Clinic, Suzuka Chuo General Hospital, Mie University Hospital.

Kyoto. Takeda Hospital, Nishijin Hospital, Kyoto University Hospital, Yakushiyama Hospital, Kyoto Min-Iren Chuo Hospital, Japanese Red Cross Kyoto Daiichi Hospital, The Japan Baptist Hospital, Aiseikai Yamashina Hospital, Shimizu Hospital, Rakusei Newtown Hospital, Daini Okamoto General Hospital, Kyoto Katsura Hospital, Saiseikai Kyoto Hospital, Nagaokakyo Hospital, Yamashiro Public Hospital, Fukuchiyama City Hospital, Maizuru Red Cross Hospital.

Nara. Hanna Central Hospital, Kura Hospital, Nara Prefectural Nara Hospital, Koseikai Takai Hospital, Tenri Hospital, Nara Medical University Hospital, Heisei Memorial Hospital, Mimuro Hospital, Nara Prefectural Gojo Hospital.

Wakayama. Wakayama Red Cross Medical Center, Wakayama Rosai Hospital, Wakayama Medical University Hospital, Wakayama National Hospital, Social Insurance Kinan Hospital, Naga Hospital.

Osaka. Sumitomo Hospital, Osaka Saiseikai Nakatsu Hospital, Osaka Kaisei Hospital, Kitaosaka Hospital, Iseikai Hospital, Yodogawa Christian Hospital, Sumire Hospital, Noe Hospital, Otemae Hospital, Osaka Red Cross Hospital, NTT Osaka Hospital, Ikuwakai Memorial Hospital, Imazato Gastroenteric Hospital, Ryokufukai Hospital, Osaka Seninhoken Hospital, Matsumoto Hospital, Kansai Denryoku Hospital, Osaka Koseinenkin Hospital, Osakakitashimin Hospital, Yamamoto Daisan Hospital, Osaka General Medical Center, Toyonaka Municipal Hospital, Senri Pain Clinic, Galacia Hospital, Minoh City Hospital, Saiseikai Suita Hospital, Suita Municipal Hospital, Osaka Medical College Hospital, Takatsuki Red Cross Hospital, Takatsuki General Hospital, Kansai Medical University Hospital, Sousei Hospital, Hoshigaoka Soseinenkin Hospital, Nozaki Tokusyukai Hospital, Katano Hospital, Kawachi General Hospital, Wakakusa Daiichi Hospital, Yao General Hospital, Fujiidera Municipal Hospital, Tondabayashi Saiseikai Hospital, Okakinen Hospital, Kinki University Hospital, Sakai Municipal Hospital, Nogami Hospital, Ueki Hospital, Osaka Rosai Hospital, NHO Kinki-Chuo Chest Medical Center, Izumi Municipal Hospital, Kishiwada Tokusyukai Hospital, Kishiwada City Hospital, Kaizuka City Hospital, Aomatsu Hospital, Izumisano Yujinkai Hospital, Higashisano Hospital, Rinku General Medical Center, Bell land General Hospital, Hanwadaini Senhoku Hospital.

Hyogo. Kobe University Hospital, Kobe City Medical Center General Hospital, Kobe Rosai Hospital, Shinko Hospital, Shakaihoken Kobe Central Hospital, Saiseikai Hyogoken Hospital, Adachi Hospital, Kaisei Hospital, Midori Hospital, Nishikobe Medical Center, Mitsubishi Kobe Hospital, Kobe City Medical Center West Hospital, Nomura Kaihin Hospital, Hayashiyama Clinic, Rokko Hospital, Rokko Island Hospital, Higashi-Kobe Hospital, Ashiya Municipal Hospital, Kansai Rosai Hospital, Okuma Hospital, Hyogo Prefectural Amagasaki Hospital, Ando Hospital, Okada Hospital, Hyogo Prefectural Tsukagoshi Hospital, Tachibana Hospital, Amagasaki Iryoseikyo Hospital, Itami City Hospital, Takarazuka City Hospital, The Veritas Hospital, Imai Hospital, Yoka Hospital, Hyogo-chuo National Hospital, Hirashima Hospital, Otsuka Hospital, NHO Himeji Medical Center, Tsukazaki Memorial Hospital, Meimai Central Hospital, Akashi Medical Center, Nishieigashima Hospital, Kakogawa West City Hospital, Kasai City Hospital, Harima Hospital, Ako City Hospital.

Tottori. Tottori Municipal Hospital, Sanin Rosai Hospital.

Shimane. Matsue Seikyo General Hospital.

Okayama. Okayama Saiseikai General Hospital, Okayama Chuo Hokancho Hospital, Tanaka Clinic, Okayama University Hospital, Japanese Red Cross Okayama Hospital, Kawasaki Medical University Kawasaki Hospital, Shigei Medical Institution Hospital, Bizen City Hinase Hospital, Okayama Rosai Hospital, Fujita Hospital, Onishi Hospital, Tamano City Hospital, Yoshino Hospital, Tsuyama Chuo Hospital, Akaiwa City Kumayama Hospital, Satou Memorial Hospital, Asano Hospital, Shigei Hospital, Kurashiki Central Hospital, Kurashiki Medical Center, Kenju Kyodo Hospital, Kasaoka City Hospital, Sakumoto Clinic, Ochiai Hospital.

Hiroshima. Chugoku Central Hospital, NHO Fukuyama Medical Center, Kobatake Hospital, Fukuyama Daiichi Hospital, JA Onomichi General Hospital, Murakami Kinen Hospital, Onomichi Municipal Hospital, Mitsugi General Hospital, Innnoshima Ishikai Hospital, Innoshima General Hospital, Mihara Medical Associations Hospital, Miyoshi Medical Association Hospital, Miyoshi Central Hospital, Nishida Clinic, Hiroshima City Hospital, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Yoshijima Hospital, Shimura Hospital, Funaki Hospital, Hiroshima City Asa Hospital, Yoshida General Hospital, Saiseikai Hiroshima Hospital, Hiroshima Park Hill Hospital, Hiroshima Prefectural Hospital, Hiroshima University Hospital, Aki City Hospital, NHO Kure Medical Center, Hakuai Clinic, Goto Hospital, Chugoku Rosai Hospital, Kure Kyosai Hospital, Kajikawa Clinic, JA Hiroshima General Hospital.

Yamaguchi. Hikari Municipal Hikari General Hospital, Hitachi Hospital, Shunan Memorial Hospital, Tokuyama Central Hospital, Hofu Gastroenteric Hospital, Hofu Onsen Hospital, Shimonoseki Kosei Hospital, Saiseikai Shimonoseki General Hospital, Yamaguchi Wakamiya Hospital, NHO Yamaguchi–Ube Medical Center, Yamaguchi Rosai Hospital, Shigeoka Hospital.

Kagawa. Kagawa Prefectural Central Hospital, Yashima General Hospital, Kagawa University Hospital, Takinomiya General Hospital, Kagawa Rosai Hospital, Miyano Hospital, Asada General Hospital, NHO Zentsuji National Hospital.

Tokushima. Tokushima Prefectural Central Hospital, Kondo Hospital, Kondo Clinic, Fujino Clinic.

Kochi. Kochi Health Sciences Center, Kochi Seikyo Hospital, NHO Kochi National Hospital, JA Kochi Hospital, Kochi Medical School Hospital, Kouhoku Hospital.

Ehime. Ehime Prefectural Central Hospital, Matsuyama Shimin Hospital, Matsuyama Red Cross Hospital, NHO Ehime National Hospital, NHO Shikoku Cancer Center, Uchiyama Hospital, Jyuzen General Hospital, Sumitomo Besshi Hospital, Ehime Rosai Hospital, Saijo Central Hospital, Manabe Hospital, Ehime Prefectural Imabari Hospital.

Fukuoka. Moji Hospital, Kyusyu Rosai Hospital, Obase Hospital, Ekiseikai Moji Hospital, Moji Rosai Hospital, Shin-Kokura Hospital, Saiseikai Yahata General Hospital, Kitakyusyu Yahata Higashi Hospital, Kyusyu Kosei Nekin Hospital, Fukuoka Shin Mizumaki Hospital, Aobadai Hospital, Shinnakama Hospital, Akimoto Hospital, NHO Kyusyu Medical Center, Hamanomachi Hospital, Nagano Clinic, Eikoh Hospital, Fukuoka Seisyukai Hospital, NHO Fukuoka Higashi Medical Center, Togo Clinic, Chidoribashi Hospital, Fukuoka City Hospital, Yagi Hospital, Kyusyu University Hospital, Fukuoka University Hospital, Muta Hospital, Fukuseikai Hospital, Fukuoka Tokusyukai Medical Center, Hakujuji Hospital, Murakami Karindou Hospital, IIzuka Hospital, Nogata Chuo Hospital, Social Insurance Tagawa Hospital, Shinkoga Hospital, St. Mary's Hospital, Yame General Hospital, Koga Hospital 21.

Saga. NHO Ureshino Medical Center, Karatsu Red Cross Hospital, Saga University Hospital, Oda Hospital, Omachi Town Hospital, Arita Kyoritsu Hospital.

Nagasaki. Iki Hospital, Saiseikai Nagasaki Hospital, Nagasaki Municipal Hospital, Uetomachi Hospital, Wajinkai Hospital, Nagasaki Memorial Hospital, Nagasaki Municipal Medical Center, Nijigaoka Hospital, Nagasaki University Hospital, St. Francis Hospital, Isahaya Health Insurance General Hospital, Omura City Municipal Hospital, Sasebo City General Hospital, Nagasaki Rosai Hospital, Sasebo Kyosai Hospital, Sasebo Chuo Hospital, Kurihara Clinic, Akagaki Naikajunkankika Clinic, NHO Nagasaki Kawatana Medical Center, Sekisyukai Hospital.

Kumamoto. Tajima Clinic, Otsuka Hospital, Yamaga Chuo Hospital, Japanese Red Cross Kumamoto Hospital, Kumamoto Chuo Hospital, Konan Hospital, Amakusa Daiichi Hospital, Amakusa Area Medical Center, Tamana Central Hospital, Kumamoto Rosai Hospital, Minamata City General Hospital and Medical Center, Taragi Hospital, Saiseikai Misumi Hospital.

Oita. Oita Cardiovascular Hospital, Oita Oka Hospital, Oita Prefectural Hospital, Usuki Cosmos Hospital, Tomachidai Surgical Hospital, Saiki Central Hospital, Nankai Hospital.

Miyazaki. Miyazaki Seikyo Hospital, Hinokage Kokuminkenkohoken Hospital, Miyazaki Prefectural Nobeoka Hospital, Nobeoka Medical Association Hospital.

Kagoshima. Yoshida Onsen Hospital, Imakiire General Hospital, Ogura Kinen Hospital, Oshima Hospital, Southern Region Hospital, Akune Citizen Hospital, Honjo Hospital, Ariake Hospital.

Okinawa. NHO Okinawa National Hospital, Adventist Medical Center, Hokubu Hospital, Okinawa Miyako Hospital.

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Exhibit L

ENVIRONMENTAL RESEARCH 23, 270–281 (1980)

93

Primary Pericardial Mesothelioma following Exposure to Asbestos

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Received December 27, 1979

INTRODUCTION

An increased incidence of malignancies after exposure to asbestos has been well established. Mesothelioma of pleura (2, 3, 7, 17, 18, 22, 25, 26), peritoneum (2), carcinoma of lung (2, 3, 7, 25, 26), larynx (3), gastrointestinal tract (2, 3), and possibly of the ovary have been described, as well as cases with multiple primary tumors (2). On the other hand, an association of asbestos exposure with primary pericardial mesothelioma has not been reported (2, 17, 18, 21). This paper describes the first case in which such a relationship existed.

CLINICAL HISTORY

This 60-year-old white male was first admitted to North Shore University Hospital on May 31, 1978 for evaluation of weight loss and left pleural effusion.

The patient was in good health until a new left bundle branch block was noted on a routine electrocardiogram, 2 years prior to admission, which progressed to complete heart block and required insertion of a permanent cardiac pacemaker. He did well until October 1977, when he developed cervical and thoracic back pains and was diagnosed to have cervical osteoarthritis. The patient's pain persisted in the left scapular region, for which he received traction, cervical collars, and anti-inflammatory agents with only partial relief.

Three weeks prior to admission, the patient began to experience easy fatigability and loss of appetite, suffering a 12-pound weight loss over 3 weeks. A routine chest x-ray film showed a left pleural effusion, and the patient was admitted to another hospital for further evaluation. There, thoracocentesis was unsuccessful, a liver–spleen scan reported as normal and the patient was transferred to North Shore University Hospital for further evaluation.

The patient was a nonsmoker and had no history of exposure to tuberculosis but had been exposed to asbestos while working in shipyards during World War II, 30 years prior to admission. The patient's medications included Darvocet for his shoulder pain and Dyazide for mild essential hypertension. There was no other history of exposure to toxic chemicals.

Physical Examination

On physical examination the patient was a well-developed, middle-aged man in no acute distress. Blood pressure was 100/70, pulse 76, temperature 37°C, respira-

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EXHIBIT L

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tion 20. The entire p
decreased breath so
the left lung, presen
edge palpable 3 cm b
was no splenomegal

Laboratory

Complete blood c
hematocrit 38.3% an
13.1 sec (control 1
Biochemical screenin
30–115), LDH 149 u
26 units/liter (0–45).
X-ray film showed a l
hemidiaphragm. The
mass in the left hilar
bital films showed no

Hospital Course

Shortly after admis
which the physician
Pleural biopsy report
placed on the skin wa
choscopy, and abdom
hepatomegaly without
During his second we
without focal neurolog
gases repeatedly sho
bicarbonate 22, pO_2 57
studies indicated a ris
liter, SGOT 309 unit
(direct 0.8 mg/dl). Ne
cephalopathy of uncer
films showed no chan
On the 17th hospital
this biopsy the patien
preference developed.
cardiac dissociation fr
few hours prior to his

The pertinent gross
diffuse on the left (Fi
plaques were noted in
pericardium weighed
tumor mass which com

owing Exposure

W. BARRETT,‡ AND

...ork University Hospital, ...s Laboratory, The Mount ...ork 10029

re to asbestos has been well ...22, 25, 26), peritoneum (2), ...intestinal tract (2, 3), and ...ases with multiple primary ...stos exposure with primary ...17, 18, 21). This paper de...sted.

...North Shore University Hos... ...nd left pleural effusion. ...idle branch block was noted ...ission, which progressed to ...manent cardiac pacemaker. ...cervical and thoracic back ...itis. The patient's pain per... ...ed traction, cervical collars,

...to experience easy fatigabil... ...oss over 3 weeks. A routine ...the patient was admitted to ...ocentesis was unsuccessful, ...nt was transferred to North

...exposure to tuberculosis but ...rds during World War II, 30 ..., included Darvocet for his ...ension. There was no other

...eloped, middle-aged man in ..., temperature 37°C, respira...

tion 20. The entire physical examination was unremarkable, with the exception of decreased breath sounds and dullness over the inferior posterolateral one-third of the left lung, presence of a cardiac pacemaker, and hepatomegaly, with the liver edge palpable 3 cm below the right costal margin with a total span of 14 cm. There was no splenomegaly, lymphadenopathy, or neurologic impairment.

Laboratory

Complete blood count on admission showed a mild microcytic anemia with hematocrit 38.3% and MCV of 78. Coagulation screening was abnormal with PT 13.1 sec (control 11.6), but PTT was 26.8 (25–35). VDRL was negative. Biochemical screening showed an alkaline phosphatase of 131 units/liter (normal 30–115), LDH 149 units/liter (60–200), SGOT 17 units/liter (10–40), and SGPT 26 units/liter (0–45). Electrocardiogram confirmed a functioning pacemaker; chest X-ray film showed a left-sided pleural effusion or thickening that obscured the left hemidiaphragm. There was also decreased volume of the left lung, a probable mass in the left hilar region, and increased density at the left heart margin. Decubital films showed no layering of fluid in the pleural cavity.

Hospital Course

Shortly after admission the patient underwent a repeat thoracocentesis, during which the physician noted a "gritty, hard area" but was unable to obtain fluid. Pleural biopsy reported only pleural fibrosis without evidence of tumor. A PPD placed on the skin was positive at 10-mm induration. Bone scan, fiberoptic bronchoscopy, and abdominal sonogram were nondiagnostic, liver scan showed mild hepatomegaly without definite abnormality, but with increased uptake in marrow. During his second week of admission, he began to develop increasing lethargy without focal neurologic signs and gradually increasing shortness of breath. Blood gases repeatedly showed a mild respiratory alkalosis with pH 7.54, pCO_2 26, bicarbonate 22, pO_2 57, oxygen saturation of 90%. At the same time, liver function studies indicated a rise in alkaline phosphatase to 210 units/liter, LDH 405 units/liter, SGOT 309 units/liter, SGPT 255 units/liter, and total bilirubin 1.5 mg/dl (direct 0.8 mg/dl). Neurology consultation suggested an evolving metabolic encephalopathy of uncertain origin and CTT of the brain was normal. Chest X-ray films showed no change.

On the 17th hospital day a liver biopsy was performed. Approximately 6 hr after this biopsy the patient became acutely confused and very lethargic. Right gaze preference developed, followed by respiratory arrest, and electrical–mechanical cardiac dissociation from which he could not be resuscitated. A chest X-ray film a few hours prior to his demise showed severe congestive heart failure.

PERTINENT AUTOPSY FINDINGS

The pertinent gross findings consisted of pleural fibrosis, focal on the right and diffuse on the left (Fig. 1A), obliterating the left pleural cavity. Large pleural plaques were noted in both hemidiaphragms (Fig. 1B). The heart together with the pericardium weighed 1400 g. The pericardial cavity was obliterated by a diffuse tumor mass which completely encased the heart and was adherent to the myocar-

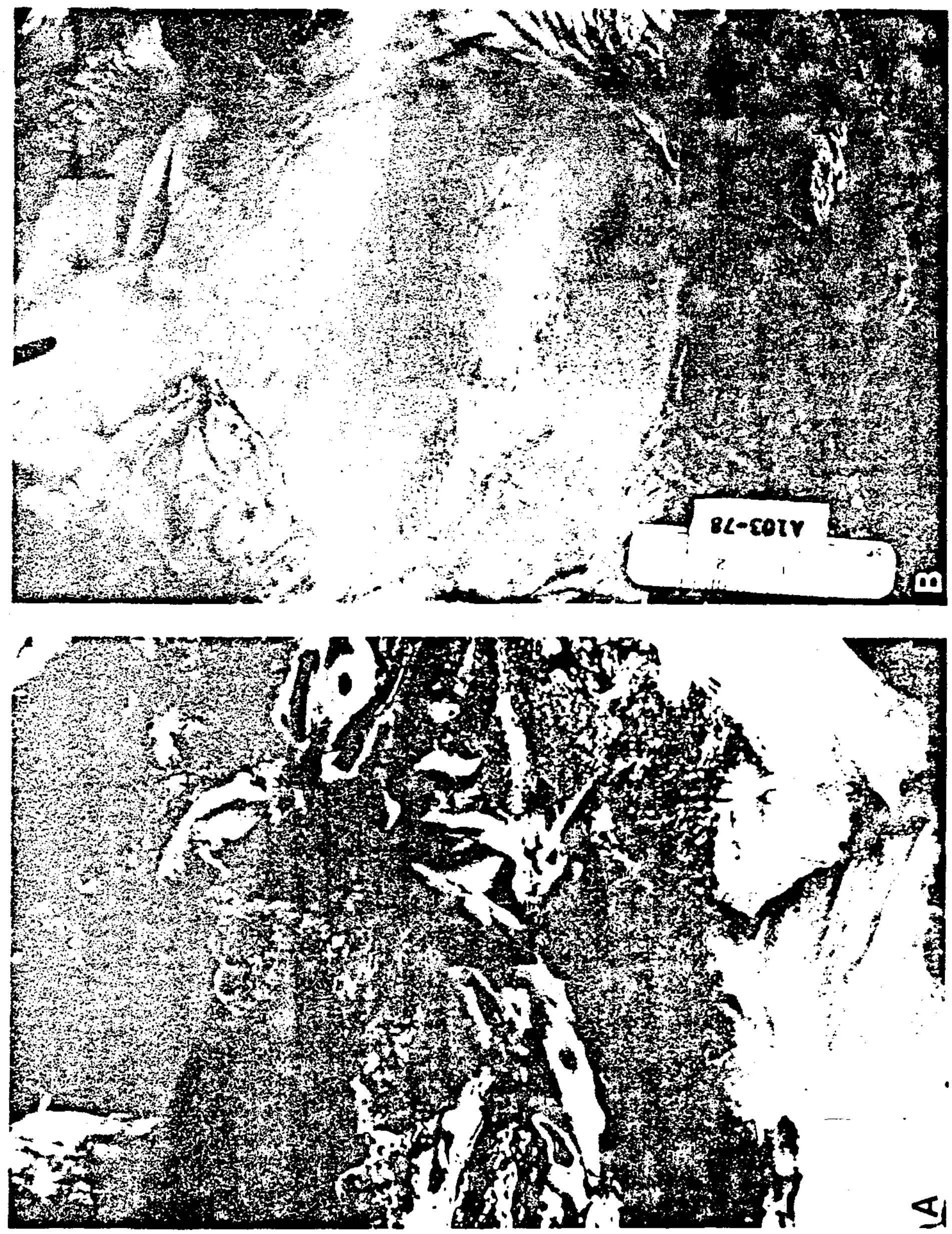
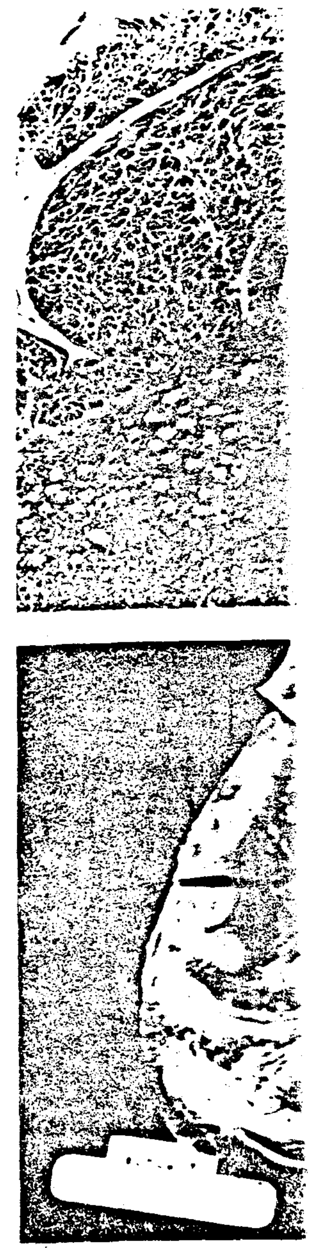


FIG. 1. (A) Diffuse pleural fibrosis. Note marked white-gray thickening of the visceral pleura, at the bottom of the picture. (B) Diaphragm with pleural plaques.



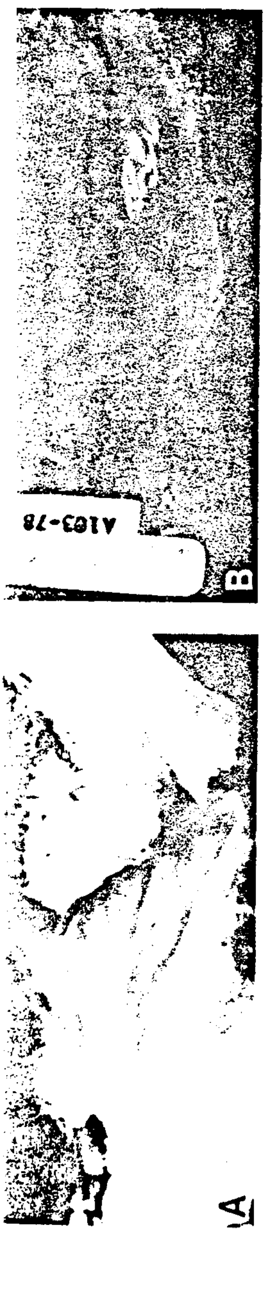
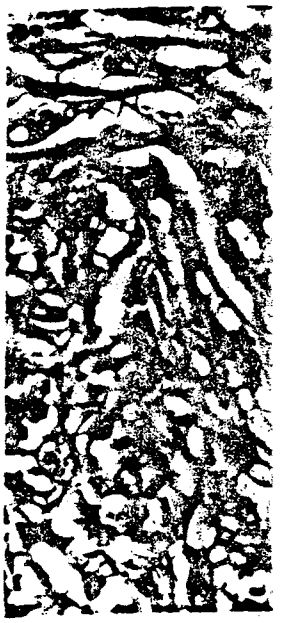


FIG. 1. (A) Diffuse pleural fibrosis. Note marked white-gray thickening of the visceral pleura, at the bottom of the picture. (B) Diaphragm with pleural plaques.

FIG. 2. (A) Pericardial mesothelioma—diffuse encasement of the heart by tumor. (B) Tumor extension into myocardium of right atrium. Hematoxylin–eosin, ×24.5.

dium (Fig. 2A). The interface of the tumor with the myocardium was generally well defined, but focally indistinct in the right atrium. The myocardium was involved by tumor with replacement up to half of its peripheral portion. Tumor encased the origin of the aorta and main pulmonary artery, as well as the terminal portion of the superior vena cava. It was focally adherent to the diaphragm, but the bulk of the tumor was restricted to the pericardium. Coronary arteries were also surrounded by tumor. The tumor was white-gray, homogeneous or nodular, rubbery, and measured between 0.2 and 2 cm in thickness. A small mural thrombus was noted in the apex of the left ventricle. The liver was congested.



Microscopic Description

The pericardial tumor was cellular with a diffuse or fascicular interlacing pattern (Fig. 3A), composed of predominantly large cells, with poorly defined pale eosinophilic cytoplasm. The nuclei were predominantly oval, moderately pleomorphic, predominantly vesicular, generally with distinct nucleoli, at times hyperchromatic (Fig. 3B). The mitotic activity varied but was never very high. Tumor cells were separated by edema and associated with collagen fibers in varying degree and a dense reticulum fiber meshwork, especially where the cellularity was decreased. Areas of necrosis were present. The tumor partially or totally obliterated the pericardial cavity. Fibrin was present in the less affected areas of the pericardium. Tumor extended in other areas to the diaphragm. On the myocardial side, the tumor infiltrated the myocardium and reached the endocardial surface, especially in the right atrium (Fig. 2B). Foci of tumor extended also into the subendocardial areas of the left ventricle, where mural thrombi were noted. In less involved areas of the epicardium the tumor surrounded large nerve trunks and coronary arteries. It extended to the adventitia of the main pulmonary artery and large vessels. The conduction system of the right atrium was sectioned and no tumor was found in the A-V node.

Lung

Ferruginous bodies were noted in both lungs. These were predominantly situated in the alveolar spaces, although they were also seen in the interalveolar septa and in perivascular and peribronchial lymphatics. The ferruginous bodies were free, or associated with polynucleated giant cells. They frequently had a coarse granular pattern. Drumstick forms, segmented bodies, elongated with needle-like forms (Fig. 4A), and dumbbell bodies (Fig. 4B) were easily found. Ferruginous bodies stained positively with iron stain. The remaining changes in the lung consisted of acute passive congestion. Recent and organizing pulmonary emboli involving medium- and small-sized vessels were found. No significant pulmonary fibrosis was noted. There was pleural fibrosis in sections from the left lung, composed of hyalinized fibrous connective tissue, in areas slightly more cellular and associated with lymphocytic infiltration. Isolated ferruginous bodies were seen within the area of pleural fibrosis. The pleural plaques noted grossly were made up of hyalinized fibrous connective tissue. There was no tumor in-

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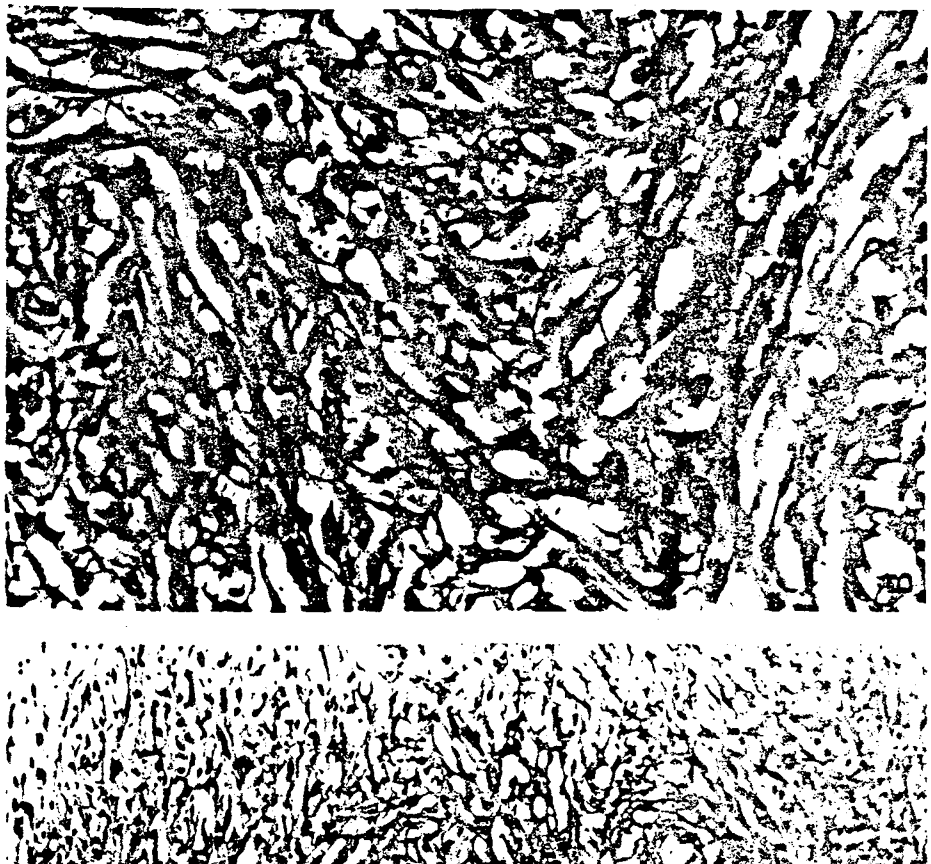

FIG. 3. (A) Pericardial mesothelioma, fibrous type. Interlacing bundles of cellular tissue. Hematoxylin eosin, ×24.5. (B) Higher magnification. Note oval-shaped, predominantly vesicular nuclei. Hematoxylin eosin, ×245.

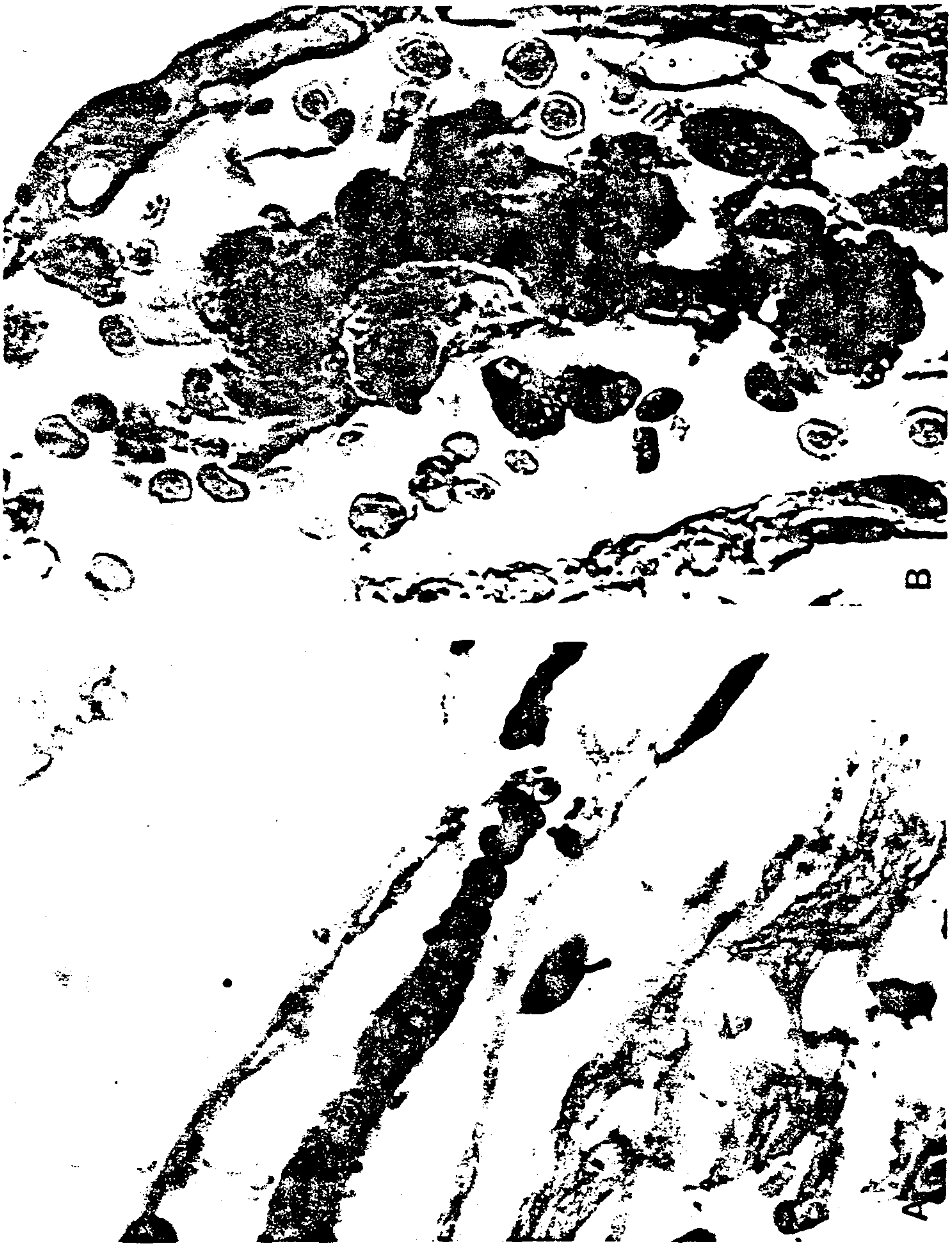

FIG. 4. Lung with ferruginous bodies. (A) Needle-shaped body in an intraalveolar space. Hematoxylin–eosin oil immersion, ×1020. (B) Dumbbell-shaped body in an interalveolar septum. Hematoxylin–eosin oil immersion, ×1020.

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FIG. 4. Lung with ferruginous bodies. (A) Needle-shaped body in an intraalveolar space. Hematoxylin–eosin oil immersion, ×1020. (B) Dumbbell-shaped body in an interalveolar septum. Hematoxylin–eosin oil immersion, ×1020.

volvement of the pleura. Fibrinous pleuritis was seen in sections from the right lung. The liver showed acute passive congestion, with severe centrilobular necrosis.

Electron Microprobe Analysis

Pulmonary parenchymal tissue was prepared for transmission electron microscopic analysis using a carbon-extraction technique. Observation of many dozens of fields at 25,000× magnification revealed a heavy to very heavy lung burden of inorganic particulates. Most of these particles were crystalline and with chemistries consistent with quartz and feldspars. Amphibole asbestos fibers, identified as amosite, were also seen (Figs. 5, 6).

DISCUSSION

This case illustrates the coexistence of a primary pericardial mesothelioma with sequelae of asbestos exposure. One might interpret these two findings as purely coincidental. We feel though that this case provides strong evidence for an asbestos-induced mesothelioma arising in the pericardium. There was a clinical

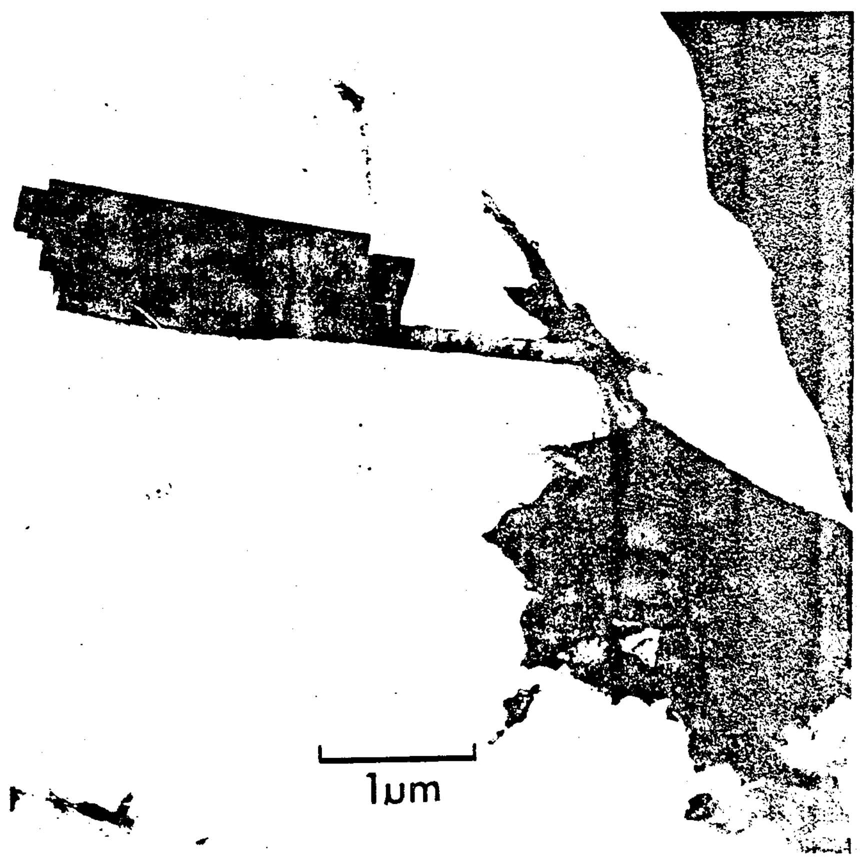

FIG. 5. Transmission electron micrograph of amphibole fiber bundle (top) in tissue (right). The amphibole variety was identified as amosite by microchemical analysis.

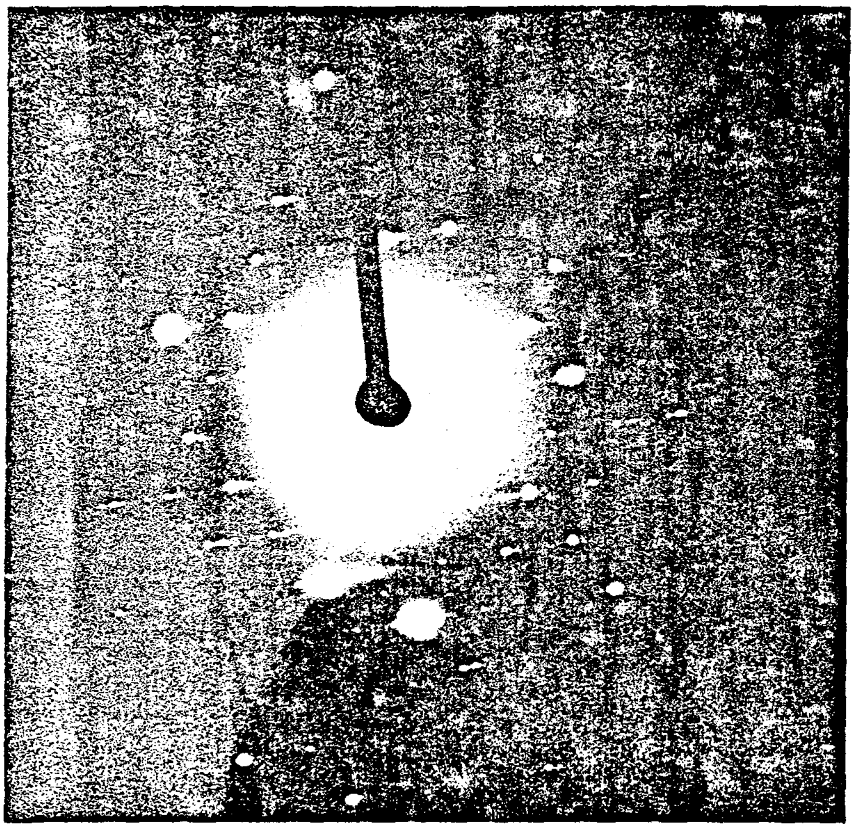

FIG. 6. Selected area electron diffraction pattern of amphibole in Fig. 5. Measurement of pattern shows 5.3-Å repeat between layer lines, characteristic of amphibole minerals.

history of asbestos exposure 30 years prior to the onset of symptoms. Ferruginous bodies were seen in the lungs, proven to contain amosite by electron microprobe analysis. This is the type of fiber frequently associated with mesothelioma.

The presence of ferruginous bodies in the lung, using optical microscopy, is indicative that asbestos might be the etiologic agent in the disease. However, two important factors should be considered when evaluating the presence of ferruginous bodies in lung tissue. It is known that hemosiderin nucleates *in vivo* on a variety of durable inorganic fibers of suitable size, including fibrous glass, ceramic fibers, talc, and other silicates. Therefore ferruginous bodies are not specific with respect to asbestos. Second, the lower limits of resolution of optical microscopy severely restricts this technique for fiber identification. On the other hand, transmission electron microscopy used in conjunction with selected area electron diffraction and electron microchemical analysis provides definitive information necessary for the identification of asbestos fiber.

By comparing the present case to large numbers of observations of lung burdens in a wide variety of circumstances, we conclude that the patient was occupationally exposed to a large amount of dust of mixed types. One of these was amosite asbestos, the type found with shipyard exposure.

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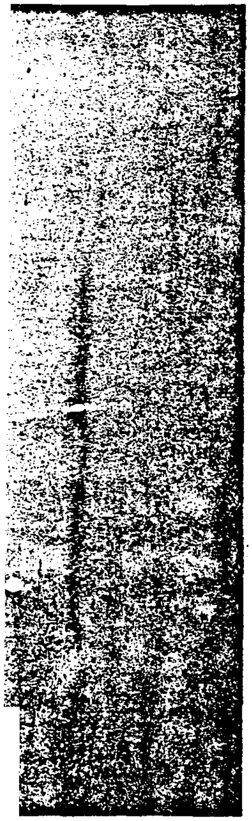

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Other morphological stigmata associated with asbestos exposure, such as pleural plaques and pleural fibrosis, were present.

The gross and microscopical features were those of a pericardial mesothelioma. No primary site could be found outside the pericardium. The pleura was extensively sampled without evidence of tumor involvement. Even though microscopical foci of tumor within the pleura can be overlooked, the bulk of the tumor situated in the pericardium would strongly suggest this as the primary site. Cohen *et al.* (5) and Pietra *et al.* (14) described a pericardial mesothelioma with focal pleural involvement and still considered the pericardium as the primary site. No tumor was found in sections of the conduction system, excluding the atrial-ventricular node as a possible primary site. Furthermore, mesotheliomas arising in the A-V node are characterized by cystic and tubular structures lined by low cuboidal epithelial cells and, hence, differ histologically from pericardial mesothelioma (9, 15). Microscopically the pericardial tumor had a sarcomatous pattern—the most common histological type associated with pericardial mesotheliomas (21).

The clinical course was that of relentless, rapidly progressing constrictive pericarditis. Signs of progressive and intractable congestive heart failure simulating constrictive pericarditis are one of the most common clinical presentations for pericardial mesothelioma (12, 21). Atrial and ventricular rhythm disturbances are usually attributed to involvement of the conduction system by tumor (8, 21). Since the conduction system in our case was not involved by tumor, the arrhythmia was probably related to coronary atherosclerosis. Death was due to low-output congestive heart failure secondary to the bulky pericardiac tumor mass, complicated by cerebral anoxia.

Compression syndromes with cough, dysphagia, and dysarthria are generally associated with bulky tumors (8). Exudative or cholesterol pericarditis has been reported (1, 8, 19). In our case the tumor was massive, obliterating the pericardial cavity, encasing the heart, and invading the myocardium. It compressed the origin of the aorta, main pulmonary artery, and superior vena cava and extended to the right atrium. Although tumor compression of the pulmonary artery and aorta is a common finding, tumor masses protruding or extending into the right atrium, superior vena cava (21), and right ventricular infundibulum rarely occur. Involvement of the coronary sinus has been described (14, 21), as well as myocardial infarction and cardiac tamponade in 26% of the cases following direct extension of tumor into the myocardium. Tumor extension into coronary arteries can lead to similar complications (21).

The main bulk of the tumor was restricted to the pericardium. Tumor extension by continuity can involve myocardium, pleura, lung, diaphragm (4, 6, 8, 14, 21), and esophagus. Metastases have been described to involve mediastinal lymph nodes, lungs, pleura (8, 14), and rarely liver (11), adrenal (14) and skin (4, 8), bone marrow and kidney (11).

The etiology of pericardial mesotheliomas is unknown. Isolated case reports refer to an association of silico-anthracosis of lung (5) or sugar cane (6). We do not believe that a pathogenic relationship has been established. In contrast to mesotheliomas of the pleura and peritoneum, asbestos has not, to the present, been implicated as an etiological agent in pericardial mesothelioma (5, 17, 18, 21).

with one exception: Churg *et al.* (4) recently described a case of pericardial mesothelioma that developed 14 years after dusting the epicardial surface with 300 mg of asbestos. This procedure was done in order to improve the cardiac circulation in a patient with angina pectoris. The patient died 2 years later. At autopsy a mixed primary pericardial mesothelioma was found that had partially obliterated the pericardial sac, extended to the myocardium, right pleura, and diaphragm, and had metastasized to the left lung and periaortic lymph nodes. Electron microprobe analysis demonstrated mainly amphibole fibers characterized as anthophyllite and tremolite asbestos admixed with a small amount of amorphous fibers, containing aluminum, silicon, and calcium interpreted as fiber glass. This case shows the capability of the pericardium to react to asbestos, with formation of mesothelioma, and bears great resemblance to ours.

Several facts concerning the relationship between asbestos and mesothelioma have been established: An increased incidence of mesotheliomas after asbestos exposure (2, 3, 7, 17, 18, 22, 25, 26) and the fact that they can be reproduced experimentally following inhalation and intrapleural or intraperitoneal inoculation (16, 23) provide evidence for a causal relationship. According to one current theory, based on statistical correlation from animal experiments, the carcinogenic effect is not so much dependent upon the fiber type or dose, but more upon its physical properties (4, 20, 24). Fibers between 10 and 80 μm and with diameter less than 2.5 μm are more carcinogenic (2, 20). Longer and thicker fibers tend to be retained less peripherally than shorter and thinner ones (10). An increased solubility of chrysotile in lung fluid has also been mentioned (10). Chrysotile fibers seem to have an increased cytotoxic effect (2, 10).

Inhalation seems to be a major pathway by which asbestos causes mesothelioma of the pleura. Migration from large bronchi to pleura is postulated with predilection to lower portions, due to gravity and respiratory movements. The pleura is reached by direct penetration or preferential drainage (2).

In the case of peritoneal mesothelioma direct penetration of the peritoneum and retrograde lymphatic flow have been suggested (10). Penetration through the gastrointestinal tract has been questioned and might occur with heavy exposure (10).

One might then postulate that the pericardium is reached by a similar route as the peritoneum, that is, direct penetration from the pleura and retrograde lymphatic flow.

It remains unclear why primary pericardial mesothelioma associated with asbestos exposure has never been described. Individual factors, such as drainage, preferential lymphatic flow, and individual biological susceptibility (2), may play a role.

ACKNOWLEDGMENTS

We are grateful to Dr. I. J. Selikoff for his valuable suggestions in preparing the manuscript, to Dr. S. Schutzer for letting us use the clinical history, and to Mrs. A. Demeter for preparation of the manuscript.

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Exhibit M

J CARDIOVASC SURG 1994;35:261-7

Primary malignant pericardial mesothelioma: A case report and review

TEJ K. KAUL, M.D., BARRY L. FIELDS, M.D., F.A.C.S., DONALD R. KAHN, M.D.

Primary malignant pericardial mesothelioma is a rare tumor of unknown etiology. The prognosis is extremely poor due to generally late presentation, inability to completely eradicate it surgically and its poor response to radiotherapy or chemotherapy. An unusual case of pericardial mesothelioma which presented as constrictive pericarditis is described. A comprehensive review of the 140 cases reported in the literature so far is presented to assist the readers in the management and prognosis of this rare, pathological tumor.

KEY WORDS: Pericardial neoplasms - Mesotelioma, malignant - Pericarditis, constrictive.

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Primary malignant pericardial mesothelioma (PMPM) is a rare tumor. The term mesothelioma was first used by Adami in 1910,[1] although the lesion was first described by Wagner in 1870.[2] Most of the reported cases have been diagnosed on autopsy.

According to Cohen [3] its incidence in 500,000 autopsies was $2.2^{10^{-1}}$. An analysis of the recent review [4-6] shows that an antemortem diagnosis was made in only 19-25% of cases. According to our estimate, 140 cases of malignant mesothelioma have been reported so far and antemortem diagnosis was made in only 40 (28.5%) cases. This report consists of a case of primary malignant pericardial mesothelioma (PMPM) and a collective review of the cases reported in the literature.

Case report

A 46-year-old white male was admitted with symptoms of congestive heart failure (CHF) and syncope for 6 weeks prior to admission. The patient also had a history of glaucoma, pseudotumor cerebri, renal failure, insulin dependent diabetes mellitus, neurogenic bladder, congenital dislocation of left hip and bilateral amputation of great toes for significant peripheral vascular disease. Of special significance was a history of asbestos exposure 27 years ago. Clinical examination revealed signs of CHF and bilateral pleural effusion.

Electrocardiogram showed normal sinus rhythm with low voltage complexes (less than 3 mm in limb leads and less than 10 mm in the precordial leads). Holter monitor revealed ventricular extrasystoles, short runs of supraventricular and ventricular tachycardia. Routine laboratory screening revealed low levels of magnesium and albumin; and elevated levels of BUN (50 mg/DL) creatinine (25 mg/DL) and alkaline phosphate (255 U/L). Chest x-ray showed an enlarged cardiac silhouette, CT scan of the chest and echocardiogram showed irregular thickening of the pericardium. On MUGA scan, left ventricular ejection fraction (LVEF) was 60%. On cardiac catheterization, right atrial pressures showed prominent X and Y decents (Fig. 1). Simultaneously recorded hemodynamic findings showed elevation and equalization of the right atrial, right ventricular diastolic, pulmonary artery diastolic, pulmonary capillary wedge pressure and the left ventricular diastolic pressures measured at 30-35 mmHg (Figs. 2A, B, C). Right ventricular traces showed the "square root" sign (Fig. 1, 2B). The diagnosis of constrictive pericarditis was made based on CT evidence of pericardial thickening and hemodynamic evidence of constrictive physiology.

The patient underwent a partial pericardiectomy on cardiopulmonary bypass to free the superior vena cava, inferior vena cava and the anterior surface of the left ventricle. The remaining pericardium was not removed because of the intramyocardial extension of the tumor and dense encasement of the heart. The pericardial biopsy specimen consisted of mul-

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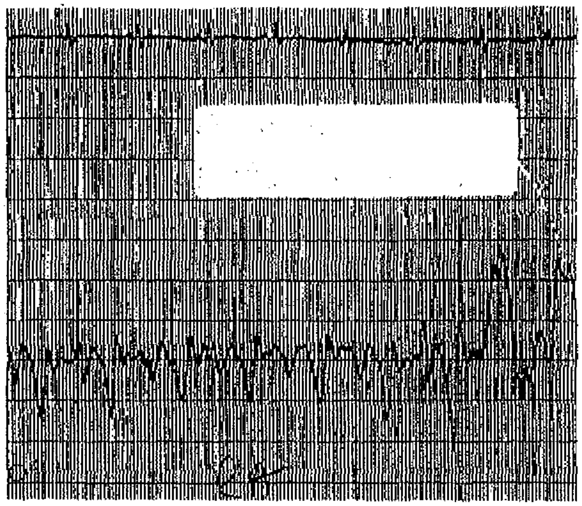

Fig. 1.—Right atrial trace showing X and Y decent. Right ventricular trace shows square root sign. Vertical scale: each line above 0 is 10 mmHg.

tiple pieces of flat pink to grey-white rubbery tissue measuring 10.0 × 7.0 × 3.0 cm in aggregate. Microscopic examination revealed malignant cells with abundant eosinophilic cytoplasm and prominent nucleoli arranged in solid cords. Occasional spindle cells surrounded by dense fibrous tissue were noted (Figs. 3A, B). Special immunohistochemical stains were employed to rule out metastatic carcinoma. These included high molecular weight keratin (HMWK). Staining was only positive for HMWK and was negative for mucin and carcino-embryonic antigen (CEA). Electron microscopic examination demonstrated cells with prominent, slender microvilli, sheets of intracellular tonofilaments and desmosomes (Fig. 4), which are diagnostic of mesothelioma. Two weeks later the patient received a course of chemotherapy (Cytoxan 1,000 mg, Adriamycin 100 mg and Carboplastin 500 mg). Subsequently, the patient's renal failure worsened requiring hemodialysis on 6 occasions and eventually the patient died of renal failure on 32nd postoperative day. Autopsy revealed 3,000 ml of sanguinous fluid in the pleural cavities and 1,000 ml in the peritoneal cavity. The heart was completely encased by grey-white rubbery tissue measuring 2.5 cm in thickness and the tumor showed superficial infiltration into the myocardium (Fig. 5). Mediastinal lymph nodes were involved but no distant metastasis were found.

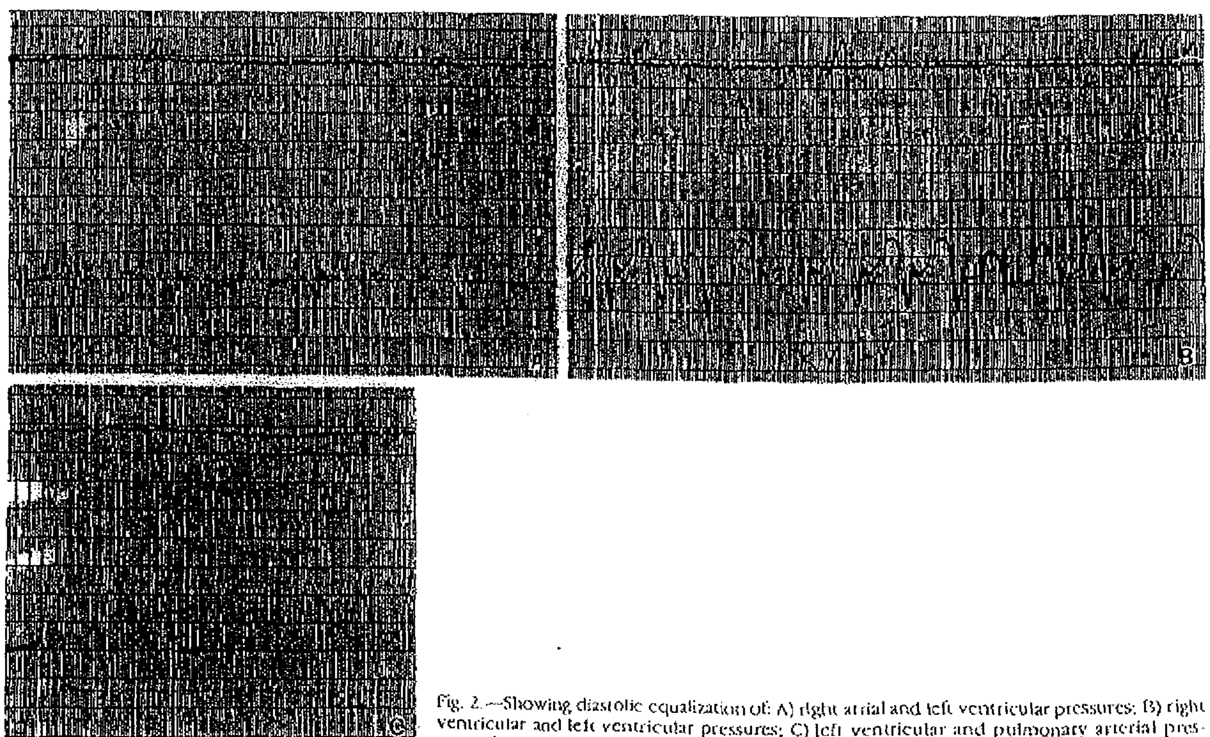

Fig. 2.—Showing diastolic equalization of: A) right atrial and left ventricular pressures; B) right ventricular and left ventricular pressures; C) left ventricular and pulmonary arterial pressures. Right ventricular and left ventricular traces also show square root sign.

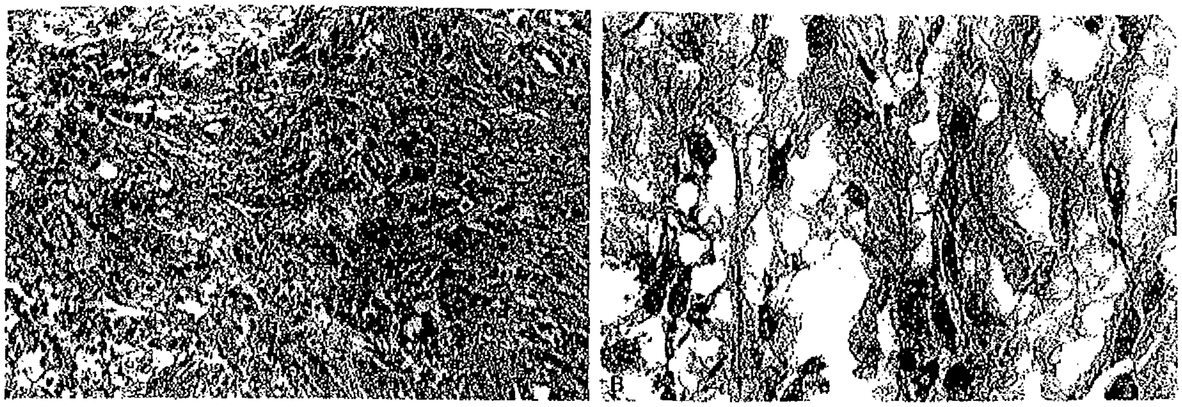

Fig. 3.—Microscopic appearance showing mixed epithelial and spindle cell elements. A) × 100; B) × 400, H E Stain.

Review of literature

Pathology

Pericardium consists of a single layer of pavement cells and a superficial fibrous layer.

Adami,[1] first showed continuity of the mesothelioma with the mesothelial pavement cell layer. Dawe [8] initially and others later have shown 3 histological subtypes of malignant mesothelioma: epithelial or tubopapillary [6-9] fibrous or sarcomatous [10 11] and mixed histology.[4 5 7 8 12 13] Pericardial mesothelioma usually has mixed epithelial and fibrous elements, whereas fibrous and epithelial elements are predominantly found in pleural and peritoneal lesions. Mesothelial cell derived malignancies do not express carcinioembryonic antigen as does adenocarcinoma.[14 15] Mesothelioma contains glycogen but the tumor is devoid of mucin and unlike adenocarcinoma is Periodic Acid Schiff (PAS) and PAS diastase negative. Mesothelioma is positive for HMWK as seen in our case. Ultrastructure may show epithelial cells with abundant microvilli,[6] elongated tonofilaments in the cystoplasm [6] and organelles including rough endoplasmic reticulum.[11] Mesothelioma may also show undifferentiated and fibroblast like cells.[15] Desmosomes may be present where the plasma membrane is intact.[6 11] Tumor may also show electron dense bodies, extracellular collagen and mucopolysaccharides.[11] Anderson *et al* in 1974 [7] have described the following criteria for the acceptance of a tumor as primary malignant mesothelioma:

1. Tumor should be strictly localized to pericardium.

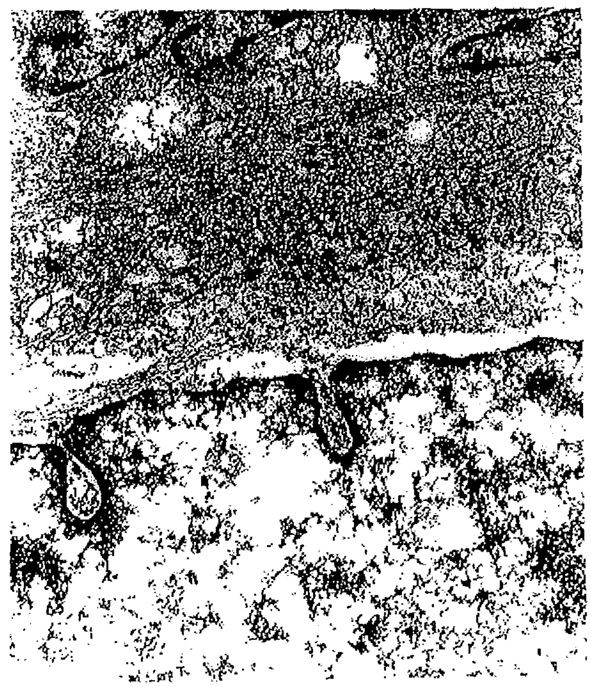

Fig. 4.—Ultrastructure showing microvilli, tonofilaments and desmosomes magnification (× 36,000).

2. It should have metastatized to lymphnodes only.
3. No other primary should be present.
4. A complete autopsy should have been performed.

Fig. 5.—Intramyocardial extension (A) and the complete encasement of the heart (B).

They however, accepted that advanced cases may show a wide spread metastases. Our case has the characteristic features of mesothelioma and it also conforms to the above requirements. As reported earlier,[12 13 16] our case also showed a complete encasement of the heart with the tumor. Malignant pericardial mesothelioma has been known to invade right atrium,[12 17] coronary sinus,[18] left atrium,[19] coronary arteries [6 20] and the conduction system. [8 18] As seen in our case it has been known to infiltrate the myocardium.[4 5 12 13 21] Unlike pleural and peritoneal mesothelioma there has been no definite correlation between asbestos exposure and the development of pericardial mesothelioma although most of the previous reports have shown no association between asbestos exposure and the development of pericardial mesothelioma.[10 13 23] In some previous reports[11 22] and in our case, a previous history of exposure to asbestos was present. The latent period after exposure in our case was 27 years; a longer latent period (30-57 years) has also been reported in the literature.[22]

Clinical presentation

Mesothelioma may remain silent for a long period. It is known to present as chest pain, [5-7 12] cough, dyspnea and palpitation,[7] superior vena caval obstruction,[6 7] cardiac tamponade,[12 21] low voltage on ECG [7 24] and CHF. [6 7 13] As in our case malignant pericardial mesothelioma may present as constrictive pericarditis.[4 5 12 13 16 25]

The most unusual presentations of PMPM have been myocardial infarction due to the invasion of the coronary arteries [6 20] and a clinical picture resembling left atrial myxoma.[19]

Diagnosis

Chest x-ray may show a non-specific cardiac enlargement, irregular cardiac contour [6 12] or an anterior mediastinal mass.[6 10 26 28] Diagnostic pneumopericardium may be used to delineate fluid levels and the tumor mass.[20 29] Collection of pericardial fluid is usually slow and large quantities may be tolerated. It has been suggested that a rapid reaccumulation of

sanguinous fluid following pericardiocentesis is highly suggestive of a neoplastic pericardial lesion.[10] Cytology of pericardial aspirate may be positive for neoplastic cells [12 13 20] but is usually unpredictable or negative [10 19] or may give false results in up to 23-27% of cases.[7 31]

Our case showed an irregular thickening of the pericardium on echocardiography. On M-mode it may difficult to distinguish a homogeneous mass from fluid.[12] Computed tomography has been found useful in the diagnosis of pericardial tumor.[10 12] Gallium scintigraphy may be used to delineate mesothelioma,[24] but it is non-specific and may also be used for the diagnosis of endocarditis, myocardial abscess, cardiomyopathy and sarcoidosis.[24] Magnetic resonance imaging has been successfully used to confirm the diagnosis of malignant mesothelioma.[27] Cardiac catheterization as in our case may reveal prominent X and Y descents in right atrial tracings and diastolic equalization of the cardiac pressures due to pericardial constriction.[23 26]

Treatment

Due to its late presentation, surgical treatment has been generally unsatisfactory. As others [4 5 7 10 11 27] we attempted a partial or subtotal pericardiectomy for the relief of symptoms. Pericardiotomy and pericardiopleurostomy have also been attempted as palliative procedures.[21] Partial pericardial resection and radiotherapy has been used with improved survival.[4 11 32] Radio-isotopes such as chromium phosphates may be used intrapericardially for the relief of symptoms.[21] Systemic chemotherapy with Doxorubicin, cyclophosphamide and Thiotepa [19] and intrapericardial Doxorubicin have also been used [20] for palliation.

Survival

According to Norman,[17] 60% of patients with malignant pericardial mesothelioma have died within 6 months of initial diagnosis. Only one case in the literature has survived up to 5 years following a partial resection and radiotherapy.[32] Only a few cases have survived up to one year after treatment.[4 11 27 33]

Discussion

Pericardial mesothelioma is a rare tumor, but paradoxically it is the most common tumor of the pericardium.[1] Primary malignant pericardial mesothelioma (PMPM) constitutes only 5% of all the mesotheliomas [34] but 50% of all pericardial tumors.[3] For a number of decades the existence of mesothelioma as a separate pathological lesion remained in dispute until Stout and Murray in 1942 [35] clearly demonstrated on tissue culture a growth of spindle cell tumor from mesothelial cells. Unlike pleural mesothelioma no definite association between a previous exposure to ashestos and subsequent development of PMPM has been established. However, few cases of PMPM reported in the literature had a previous ashestos exposure.[22] On reviewing the literature it appears that approximately 72% of all PMPM were actually diagnosed on autopsy (Table I). This obviously indicates a silent nature of this highly malignant and lethal tumor. More recently, especially since Andersson's review, there has been a greater awareness of PMPM clinically and antemortem diagnosis has been made through the detection of anterior mediastinal mass and irregularity of the cardiac silhouette on chest x-ray, echocardiography, CT scanning and magnetic resonance imaging. However, surgical intervention for PMPM which appears to have been attempted only in a very limited number of patients (Table I) has remained unsatisfactory, most probably due to its late presentation and especially due to its deep often dense almost placental like infiltration of the myo-

TABLE I.--*Recent reviews, report of primary malignant pericardial mesothelioma.*

Authors (year)	Cases n*
Chun *et al.* (1980)	120 from literature + 1
Cusumo (1980)	1
Vlling (1982)	1
Beck (1982)	5
Yogelzang (1984)	2
Nishikata (1987)	1
Gossinger (1988)	1
Llewellyn (1987)	1
Fokuda (1989)	1
Aggarwal (1991)	1
Present case	1

*72% were diagnosed on autopsy

TABLE II.—*Surgically treated cases of PMPM in the literature.*

Authors (year)	No. of cases	Age (year)	Sex	Presentation	Histology	Treatment	Survival (months)
Van de Water (1967)	1	43	M	Constrictive pericarditis	Spindle cell	Bilateral anterior thoracostomy/partial pericardiectomy & radiotherapy	4 1/2
Styman (1971)	2	17	M	Constrictive pericarditis	Spindle cell	Thoracotomy & partial pericardiectomy	19
Anderson (1974)	7	—	—	—	—	—	75, 3, 7, 8, 12 & 60
Cusmano (1980)	1	63	M	Cardiac camponade	Spindle cell	Pericardiocentesis Intrapericardial Doxorubicin	2
Llewellyn (1987)	1	69	M	Constrictive pericarditis	Mixed	Left thoracotomy	Same day
Lund (1987)	1	32	M	Left atrial mixoma	Malignant mesothelial cells	Thoracotomy, partial pericardiectomy, radiotherapy, chemotherapy with cyclophosphamide, Doxorubicin	4
Gossinger (1988)	1	73	F	Pericardial mass	Spindle cell	Left thoracotomy, partial pericardiectomy	5
Fukuda (1989)	1	51	F	Anterior mediastinal mass	Spindle cell	Partial pericardiectomy on block removal	10
Aggarwal (1991)	1	30	M	Anterior mediastinal mass	Spindle cell	Partial pericardiectomy mediansternotomy	Not given
Present case (1993)	1	46	M	Constrictive pericarditis	Mixed	Partial pericardiectomy on CPB	1

cardium.[5 23 27] In all the cases treated so far, surgical eradication of the tumor was found impossible.[4 5 7 13 23 27] However, some relief was obtained in the patients who presented with constrictive pericarditis.[4 5 12 13 16 23 25] Radiotherapy, systemic or intracavitary chemotherapy have not shown any significant impact on the course and the prognosis of the PMPM.[21 34] It appears that at this stage, it is still difficult to detect and treat PMPM at an early stage. Only one case of PMPM treated with surgical resection and radiotherapy has survived for 5 years.[32] The average survival of the cases reviewed by us was 10 months which is marginally better than an average of 6 months reported previously.[37]

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Exhibit N

Br Heart J 1987;57:54–7

Pericardial constriction caused by primary mesothelioma

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SUMMARY Primary pericardial mesothelioma is an extremely rare tumour. This case illustrates the typical late presentation with symptoms and signs of constrictive pericarditis. An unusual feature was complete encasement of the heart by tumour. No satisfactory treatment is available.

Case report

A 69 year old retired seaman gave a two month history of progressively worsening breathlessness and central chest discomfort on effort. There was associated weight loss, anorexia, night sweats, and pronounced peripheral oedema. Symptoms were partly relieved by diuretics and vasodilators.

Previous illnesses included longstanding, but well controlled, hypertension (treated with atenolol and hydralazine) and resection of an enlarged prostate two years before presentation, in which histological examination showed foci of well differentiated prostatic adenocarcinoma.

On examination he was unwell, slightly jaundiced, and dyspnoeic at rest. The pulse was regular and of small volume, and the blood pressure was 90/60 mm Hg. The jugular venous pulse was elevated to the angle of the jaw, with sharp "y" descent and there was pitting oedema up to the knees. The cardiac apex was impalpable and the heart sounds quiet without added sounds or murmurs. The lungs were moderately congested with a small right pleural effusion.

A chest radiograph showed cardiac enlargement (cardiothoracic ratio 58%), pulmonary venous congestion, linear collapse, and bilateral pleural effusions. No pleural plaques or lung tumour were seen. The electrocardiogram showed low voltage QRS complexes with non-specific T wave changes. Echocardiography demonstrated thickened pericardium with small anterior and posterior pericardial effusions, normal valves without vegetations, and satisfactory ventricular contractility.

Initial investigation showed: haemoglobin 12·8 g/dl; white cell count 10·8 × 10^9/l; sodium 128 mmol/l; urea 21·7 mmol/l; creatinine 211 mmol/l; concentrations of liver transaminases were slightly raised; lactate dehydrogenase 189 U/l (normal 80–160 U/l); alkaline phosphatase 133 U/l. Blood, sputum, and urine culture were sterile. Viral titres, tuberculin test, and autoantibody screen were negative. Acid phosphatase concentration was not raised. Pleural aspiration drew clear yellow fluid, protein content 35 g/l; cytology showed mesothelial cells.

At cardiac catheterisation diastolic pressures in all chambers were almost equal (right atrium mean 22; right ventricle 40/22; pulmonary artery 45/30; mean pulmonary capillary wedge 24; left ventricle 100/22; aorta 100/80 mm Hg.) Cineangiography showed small and well contracting left and right ventricles with considerable limitation of diastolic filling. Coronary arteriography showed a 75% proximal stenosis of the anterior descending branch of the left coronary artery.

At thoractomy the heart was found to be surrounded by thick white tumour masses, which had spread to the adjacent mediastinal nodes and pleura. Resection was impossible and the patient died several hours after return from the operating theatre.

At postmortem the whole heart was found to be encased by tumour, with pericardium adherent to the epicardium forming a continuous band of white tissue 1 cm thick (fig 1). Several nodules of tumour surrounded the great vessels and the heart plus tumour weighed over 1 kg. Numerous small pleural nodules of tumour were present, and none was

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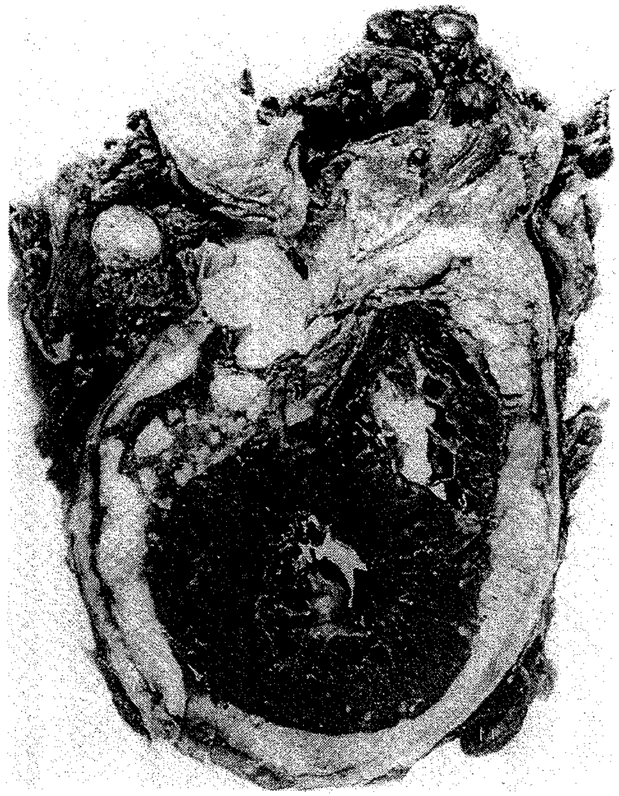

Fig 1 *Transverse section of heart and great vessels showing complete encasement of heart by tumour.*

larger than 1 cm in diameter. There was no evidence of residual prostatic tumour or of any pelvic or abdominal neoplasia or lymphadenopathy.

The histological appearances of most of the pericardial tumour indicated an anaplastic carcinoma that had infiltrated the myocardium (fig 2). There were, however, numerous areas showing papillary differentiation (fig 3a), acinar elements (fig 3b), mesothelial differentiation (fig 3c), and frankly sarcomatous differentiation (fig 3d). Neutral mucin stains (periodic acid Schiff diastase) and immunoperoxidase stains for prostatic acid phosphatase, prostatic specific antigen, and carcinoembryonic antigen were negative. These features meet the diagnostic criteria for malignant mesothelioma.[1]

Discussion

Primary tumours of the pericardium are extremely rare; one of the largest necropsy series of recent years gives an incidence of 0·0022% in 500 000 cases.[2] Mesothelioma is probably the commonest type, followed by sarcoma, teratoma, fibroma, lipoma, and angioma.[3] The incidence in both sexes is almost equal, with an age range of 1–79 years.

The tumour is commonly diagnosed at a late stage and often results in evidence of constriction caused by tumour expansion or associated serous or haemorrhagic pericardial effusion. The diagnosis in this case was suspected because of thickened pericardium seen on the echocardiogram; and in view of the previous history of malignancy, a secondary rather than primary tumour seemed more likely. Although cardiac catheterisation can confirm pericardial constriction and indicates ventricular function, definitive diagnosis is often not made until thoractomy. The pleural exudate obtained in this case contained non-specific mesothelial cells and was clearly a pointer to the tumour. Pericardial aspiration was not attempted before operation but would probably have produced a dry tap, perhaps a helpful feature in differential diagnosis.

Radioisotope scanning with gallium or technetium may be used to detect malignant pericardial effusion but results are not regarded as being highly specific.[3] Computed tomography may well be helpful in distinguishing tumour from fluid in the pericardial space.[4]

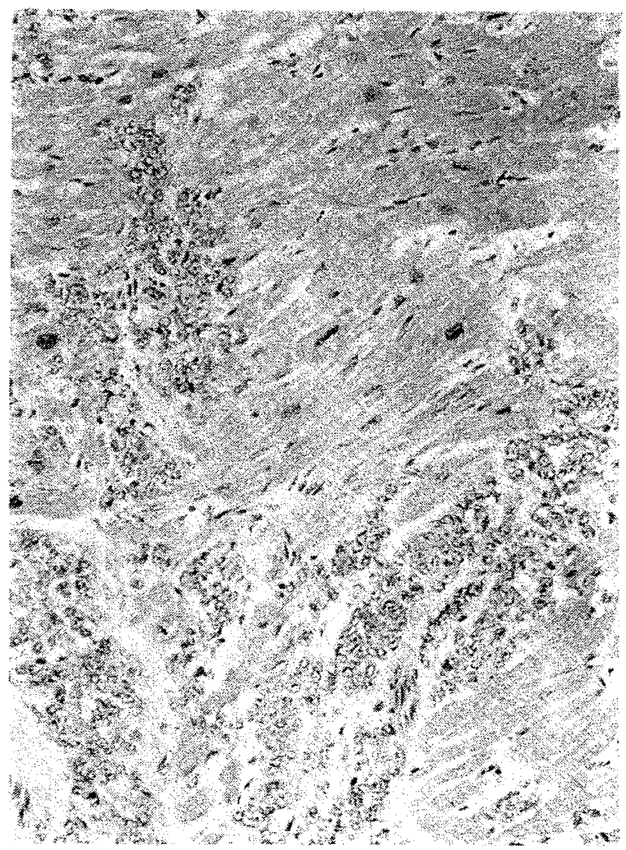

Fig 2 *Photomicrograph showing myocardial infiltration by tumour.*

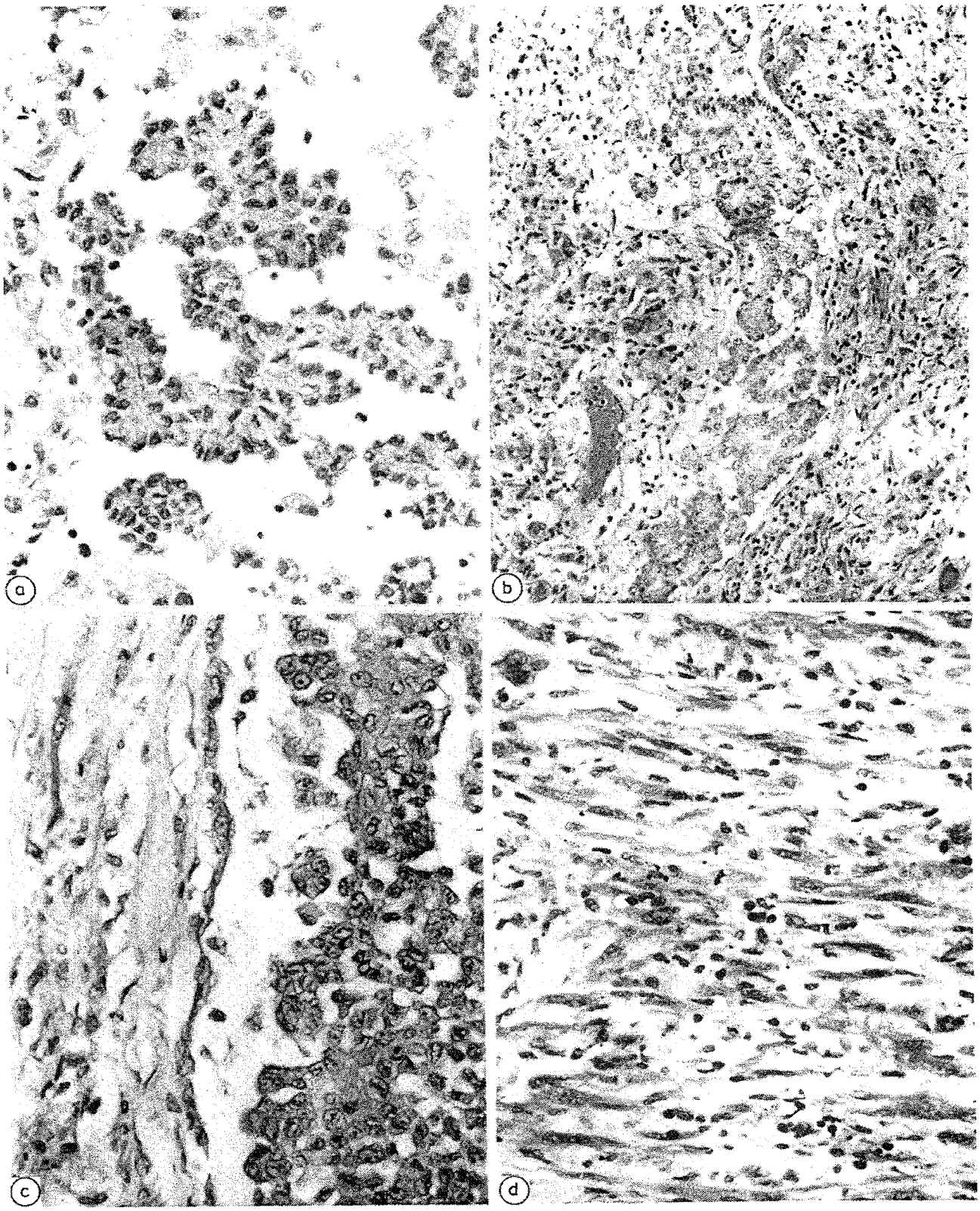

Fig 3 *Photomicrographs (a) papillary elements, (b) acinar elements, and showing (c) mesothelial and (d) sarcomatous differentiation.*

The gross pathological appearance may be of a localised mass, solid or cystic or angiomatous, or of diffuse nodules. Complete encasement of the heart by tumour is an unusual feature and has been rarely reported.[4-8] The pericardial tumour is often adherent to or may invade the myocardium.[9] The tumour can also invade the conducting tissue or coronary arteries, or compress the great vessels. Local spread is common, but extrathoracic metastasis is extremely rare. In one review stringent criteria were applied to the diagnosis of mesothelioma when the pericardium was the postulated primary site,[10] particularly when numerous pleural metastases were present. The clinical presentation and pathological distribution of tumour must, therefore, be considered together when deciding on the diagnosis and primary site. Histological differentiation is often difficult because of the pleomorphic nature of the tumour.

Possibilities for treatment are usually limited by late detection. Complete tumour resection is virtually impossible so operation is usually confined to attempts to relieve obstruction. The results of systemic chemotherapy are disappointing; but if associated pericardial effusion is present local instillation of cytotoxic drugs or sclerosing agents can be helpful. Radiotherapy may temporarily reduce the size of the tumour.

There has been no clear association between asbestos exposure and pericardial mesothelioma. This is probably because the paucity of recorded cases has not allowed an adequate epidemiological study. As in many cases, this diagnosis was made after death and the relevant history was not available.

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Exhibit O

Incidence of extrapleural malignant mesothelioma and asbestos exposure, from the Italian national register

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ABSTRACT

Objectives The epidemiology of extrapleural malignant mesothelioma is rarely discussed and the risk of misdiagnosis and the very low incidence complicate the picture. This study presents data on extrapleural malignant mesothelioma from the Italian National Mesothelioma Register (ReNaM).

Methods ReNaM works on a regional basis, searching for cases and interviewing subjects to investigate asbestos exposure. Classification and code criteria for certainty of diagnosis and exposure modalities are set by national guidelines. Between 1993 and 2004, 681 cases were collected. Incidence measures and exposure data refer to the ReNaM database. Age-standardised rates were estimated by the direct method using the Italian resident population in 2001. Correlations between the incidence of pleural and non-pleural malignant mesothelioma for the 103 Italian provinces were analysed.

Results Standardised incidence rates (Italy, 2004, per million inhabitants) were 2.1 and 1.2 cases for the peritoneal site (in men and women, respectively), 0.2 cases for the tunica vaginalis testis, and 0.1 in the pericardial site, varying widely in different parts of the country. Mean age at diagnosis for all extrapleural malignant mesothelioma cases was 64.4 years and the men/women ratio was 1.57:1. Median latency was over 40 years for all extrapleural sites combined. The correlation between pleural and peritoneal mesothelioma was 0.71 (Pearson's r coefficient, $p<0.001$). Modalities of exposure to asbestos fibres were investigated for 392 cases.

Conclusions The rarity of the disease, the low specificity of diagnosis and difficulties in identifying the modalities of asbestos exposure call for caution in discussing aetiological factors other than asbestos.

What this paper adds

- The epidemiology of non-pleural malignant mesothelioma, whose incidence is very low, is rarely discussed, and the diagnosis is still limited by poor sensitivity and specificity, with a particularly high risk of misdiagnosis in women.
- Recently it has been suggested that the association between asbestos exposure and peritoneal mesothelioma is weaker than for the pleural form, but the rarity of the disease, the low specificity of diagnosis and difficulties in identifying the modalities of asbestos exposure call for caution in discussing the role of aetiological factors other than asbestos.
- Between 1993 and 2004, 681 cases of non-pleural mesothelioma in Italy were collected through a national registration system, with individual recognition of asbestos exposure, applying standardised methods for diagnosis and exposure modalities.
- Exposure to asbestos was the most important risk factor, so the possibility of a weaker relationship needs to be carefully considered, depending on the misdiagnosis of other malignancies (especially in women) and on the difficulty of clearly identifying occupational exposure in non-industrial work settings, as well as non-occupational exposure, both of which are important for women.

INTRODUCTION

Malignant mesothelioma (MM) is a lethal tumour generally induced by exposure to asbestos. It arises from the serous membranes of the pleura and, less frequently, of the peritoneal and pericardial cavities and from the tunica vaginalis testis. The epidemiology of extrapleural malignant mesothelioma (EPMM) is rarely discussed. The European population-base cancer registries published figures for the incidence of peritoneal mesothelioma, which ranged between 0.1 and 0.25 cases (per 100 000 inhabitants) for men and 0.05 and 0.1 for women in 2001.[1] The United States SEER program reported incidence rates of 0.12 for men and 0.08 for women in 1973–2005 with no significant temporal trend in that period,[2] and predicted a ratio of 6.3 between pleural and peritoneal cases in the USA in the decades up to 2050.[3] Cases of pericardial and testicular mesothelioma are only sporadically reported and it is not easy to establish their extent and the aetiological characteristics from the epidemiological point of view.[4]

The differences between male and female incidence rates suggest that diagnosis of peritoneal MM still suffers poor sensitivity and specificity and the risk of misdiagnosis is particularly high for women with neoplasms from abdominal organs, above all primary peritoneal serous carcinoma and

ovarian serous carcinoma.[5] The disease rarely presents as ovarian masses although ovarian mesotheliomas have been described.[6] Pleural/peritoneal incidence ratios vary widely in published studies, but generally the incidence of peritoneal mesothelioma is one order of magnitude lower than pleural forms.[7]

The relationship between MM and asbestos exposure has been definitely demonstrated, although some aspects of the biological causal mechanisms are still not clear. A lower attributable risk to asbestos for peritoneal than pleural MM, has been suggested, in the light of the lower men/women ratio in EPMM.[8] Taking account of fibre type, a dose–effect relationship with asbestos exposure has been shown for peritoneal MM, but with a distinct shape compared with pleural cases.[9] Moreover, the peritoneal MM risk rises with duration of exposure and latency, much more steeply than for the pleural form.[10]

Italy was one of the main raw asbestos-producing and asbestos-importing countries until the ban in 1992. A permanent MM epidemiological surveillance system has in fact been operative since 1993 (and was made mandatory in 2002), based on a national MM register (ReNaM) established at the National Institute for Occupational Safety and Health (ISPESL), which publishes figures for incidence, survival and asbestos exposure.[11–13] This paper presents the current data on EPMM from ReNaM.

METHODS

ReNaM has a regional structure: regional operating centres (COR) have been gradually established in 18 of the 19 Italian regions and one of the two autonomous provinces, covering almost the whole country (98.5% of the Italian population). The Italian mesothelioma register has been extensively described elsewhere.[11 14]

Occupational and residential history, and lifestyle habits, are obtained using a standardised questionnaire administered by a trained interviewer to the subject or next of kin. At present 6640 MM cases (out of 9166, 72.4%) and 437 EPMM cases (out of 681, 64.2%) have been interviewed. Occupational exposure is classified qualitatively, as definite, probable or possible. Definite occupational exposure refers to people whose work involved the use of asbestos or materials containing it. Probable exposure is attributed to those who have worked in a firm or sector where asbestos was certainly used, but whose exposure cannot be documented, and possible exposure to people who have worked in a firm or sector where asbestos might have been used.

At present the ReNaM has collected cases with a diagnosis of MM in the period 1993–2004. Italian regions, through each COR, did not all contribute equally during this period: Piedmont, Veneto, Tuscany and Apulia produced incidence regional case lists starting from 1993; Liguria, Emilia-Romagna and Marche from 1996; Sicily from 1998; Friuli Venezia-Giulia and Valle D'Aosta from 2000; and Campania from 2001. Lombardy has produced incidence figures for 2000 and 2001, but more recent data are still awaited. As yet, case lists from Lazio, Abruzzo, Basilicata, Calabria and Sardinia cannot be considered complete. Finally, Umbria, Molise and the two autonomous provinces of Trento and Bolzano did not collect any data at all.

Incidence measures refer to the space-territory coverage according to incidence data collection, as specified above. Age-standardised rates were estimated by the direct method using the Italian resident population in 2001. The men/women ratio for pleural and extrapleural case lists and the pleural/extrapleural ratio were calculated. The correlations between pleural and non-pleural MM incidence for each of the 103 Italian provinces were analysed, then stratified by sex. We estimated the person/years (PY) of observation, the number of pleural, peritoneal, pericardial and testicular MM cases and the crude rate for all provinces, with the Pearson's r correlation coefficient for all pairs of anatomical sites, and tested the statistical significance of these correlations. Finally, we calculated the crude rate of EPMM for the 8101 Italian municipalities. Exposure data refer to the whole ReNaM database. To provide an indication of the size of the industry, we estimated the cumulative population at risk for the branches of industry with a relevant number of EPMM cases. The initial size of the population was determined from the workforce at national census in 1961 and an annual change yearly was added (or detracted) depending on intercensual differences. All statistical analysis was carried out with SPSS software V.17.

RESULTS

We collected a case list of 9166 MM cases between 1993 and 2004. The pleural site was reported for 92.6% of the MM cases (8485), and peritoneal sites for 6.7% of all cases (614); pericardial and testicular sites accounted for, respectively, 0.4% and 0.3% (36 and 31 cases). The pleural/non-pleural ratio is 12.5/1 (13.8/1 for the peritoneal site) with no significant changes between 1993 and 2004. The men/women ratio is higher in the pleural group (2.75:1) than the extrapleural (1.57:1). Table 1 shows the distribution by sex, age, incidence period, diagnostic certainty, morphology and modalities of interview for 681 incident EPMM cases.

EPMM is a very rare disease, with standardised incidence rates for the peritoneal site of, respectively, 2.1 and 1.2 for men and women (cases per million inhabitants, Italy, 2004). It is more frequent in the tunica vaginalis testis (0.2 cases per million) than in the pericardium (0.1 per million in men and women). When restricted only to certain mesothelioma, the incidence rate for peritoneal MM is about 20% lower for both men and women.

The pooled national figures are determined by regional incidence which varies widely. Certain, probable and possible peritoneal incidence go from 0.9 per million inhabitants (Veneto and Apulia) to 6.1 (Piedmont) for men and from 0 (Valle d'Aosta, Friuli Venezia-Giulia, Liguria, Campania, Apulia, Sicily) to 3.9 (Piedmont) for women. Liguria and Friuli Venezia-Giulia have high rates for peritoneal mesothelioma in men (respectively, 5.5 and 4.9), but there were no female cases in 2004. The wide variability of EPMM cases among Italian municipalities is illustrated in figure 1.

Distribution by age differed significantly among anatomical sites. Cases $<$45 years accounted for only 1.9% in the pleural group, 3.1% in the peritoneal, 11.1% in the pericardial, and 3.2% in the group of testicular MM. In the 25–44-year age group, there were 31 recorded peritoneal cases (as opposed to 15 expected) assuming equal distribution (χ^2 test 264.7; $p<0.001$). Mean age at diagnosis for the overall EPMM cases was 64.4 years (range 18–95) for males and 65.4 years (range 19–94) for females, younger than for pleural MM (respectively, 68.3 years, range 22–97, and 69.5 years, range 30–103).

We interviewed 64.2% of EPMM cases (437/681) to identify their exposure modalities, and 73.1% of pleural MM (6203/8485) (table 2). Just over two-thirds of the peritoneal cases (69.7%, 276/396) had had occupational, environmental, household, or leisure-related exposure, compared with 81.3% of pleural MM with some form of exposure (5040/6203 cases). The proportion was much lower for women (53.1%), and for the combined male/female pericardial group (61.9%). In the latter far more men had a history of exposure (91.7%) than women (22.2%).

EXHIBIT O

Table 1 Non-pleural malignant mesothelioma (MM) cases collected in the Italian national mesothelioma register (ReNaM) by anatomical site, sex, age, period of diagnosis, level of diagnostic certainty, morphology and modalities of interview, Italy, 1993–2004

	Peritoneum	Pericardium	Tunica vaginalis testis	All non-pleural sites
Sex, n (%)				
M	362 (59)	23 (63.9)	31 (100)	416 (61.1)
F	252 (41)	13 (36.1)	–	265 (38.9)
Age, years, n (%)				
0–44	34 (5.5)	6 (16.7)	5 (16.1)	45 (6.6)
45–64	247 (40.2)	12 (33.3)	5 (16.1)	264 (38.8)
65–74	215 (35)	13 (36.1)	10 (32.3)	238 (34.9)
75+	118 (19.2)	5 (13.9)	11 (35.5)	134 (19.7)
Period of diagnosis, n (%)				
1993–1996	104 (16.9)	5 (13.9)	6 (19.4)	115 (16.9)
1997–2000	205 (33.4)	11 (30.6)	5 (16.1)	221 (32.4)
2001–2004	305 (49.7)	20 (55.6)	20 (64.5)	345 (50.7)
Diagnostic certainty, n (%)				
MM certain	491 (80)	28 (77.8)	31 (100)	550 (80.8)
MM probable or possible	123 (20)	8 (22.2)	–	131 (19.2)
Morphology, n (%)				
Epithelioid	328 (53.4)	10 (27.8)	15 (48.4)	353 (51.8)
Biphasic	53 (8.6)	7 (19.4)	5 (16.1)	65 (9.5)
Sarcomatous	17 (2.8)	2 (5.6)	2 (6.5)	21 (3.1)
MM NAS	167 (27.2)	10 (27.8)	9 (29.0)	186 (27.3)
Not available	49 (8.0)	7 (19.4)		56 (8.2)
Investigated by interview, n (%)				
Direct	152 (24.8)	6 (16.7)	10 (32.3)	168 (24.7)
Indirect	199 (32.4)	14 (38.9)	10 (32.3)	223 (32.7)
No interview	263 (42.8)	16 (44.4)	11 (35.5)	290 (42.6)
Total	614 (100.0)	36 (100.0)	31 (100.0)	681 (100.0)

NAS, not otherwise specified.

The economic sectors and the activities involving exposure to asbestos, their sizes in terms of PY and estimated risks by sector are reported in table 3.

Half the peritoneal MM cases had been exposed to asbestos in the asbestos-cement industry, shipbuilding and repair, heavy industry or construction sectors. The proportion of exposures in the asbestos-cement industry was higher for peritoneal (21.8% of total occupationally exposed subjects) than for pleural MM (4.0%). Figures were similar in the textile industry (9% for peritoneal and 6.3% for pleural) with a higher prevalence of females for peritoneal (75%) than pleural forms (58%).

Mean latency (defined as the time elapsing between the beginning of exposure to asbestos and diagnosis) was estimated for cases with sufficient information and was, respectively, 43.6 and 40.8 years for peritoneal MM in men and women. For the 10 men with pericardial MM it was 41.7, and 46.8 for the 13 cases with the testicular form.

There was a close correlation between pleural and peritoneal mesothelioma in the Italian provinces, with high statistical significance (Pearson's r coefficient 0.71; $p<0.0001$) even though some had high pleural rates but peritoneal rates lower than expected (figure 2). After analysis separately by sex, the correlations remained clearly significant (r 0.63 for men and 0.65 for women; $p<0.0001$ for both). The correlation was weaker between pleural and pericardial and testicular mesothelioma, and did not reach statistical significance ($p=0.18$ for both comparisons).

DISCUSSION

The ReNaM is one of the largest systems of epidemiological surveillance for malignant mesothelioma in the world. Case lists of peritoneal mesothelioma from a national registration system with identification of individual asbestos exposure modalities are not frequently published, and reports of pericardial or testicular cases are merely episodic. The Italian national programme of MM epidemiological surveillance covers a large part of the country (>340 million PY of observations) including some areas where there was substantial direct use of asbestos in shipbuilding, railway stock construction and maintenance, the asbestos-cement industry, and other industrial settings entailing ample exposure to asbestos in more recent years.

Some limitations regarding the ReNaM dataset need to be discussed. The registry has not been developed uniformly throughout the country. As stated in the Methods, some regions started collecting figures for the incidence even before the national register was set up in 1992; others started later and some are still not participating. Any attempt at assessing the pattern of MM incidence is therefore limited, particularly for EPMM, considering their small numbers. At present it is not possible even to estimate or compare the patterns of pleural and extrapleural mesothelioma incidence. Furthermore, the regions examine asbestos exposure at different levels, depending on their resources and knowledge. The national guidelines intended to standardise the collection of mesothelioma cases do help even out the imbalances, but many of the marked differences between regions persist. In addition, differences in diagnostic procedures make it harder to ensure complete detection for EPMM, and the acceptance of EPMM cases not backed by histological diagnosis implies a larger risk of false-positives than for pleural MM.

The substantial proportion of patients who were not interviewed (35.8% of EPMM cases) is another critical point of the surveillance system. When a long time elapses between diagnosis and the alert to the regional centre, proceeding with the interview becomes more difficult so closing this gap is important. Then too, considering the poor prognosis for this disease it is understandable that an appreciable number of patients will refuse interviews.

The epidemiology of EPMM is complicated by the low sensitivity and the poor specificity of the diagnosis. It is often hard to make a histological distinction between peritoneal epithelioid mesotheliomas and serous carcinomas diffusely involving the peritoneum, though immunohistochemistry and electron microscopy help clear up doubts; a clear distinction could be made between these two malignancies in all cases in which electron microscopy was carried out.[15]

The female component was particularly high for EPMM, with male/female ratios of 1.4:1 and 1.9:1 for peritoneal and pericardium, respectively, and there were 2.7 male pleural MM cases for each female in ReNaM, in agreement with other published case lists.[16]

Pericardial and peritoneal MM systematically have a worse prognosis (median survival 5–6.9 months) than pleural MM (median survival 7.9–10 months). A recent analysis found shorter survival for the most peritoneal MM cases, but at the same time a larger proportion of long-term survivors among these patients: longer survival was associated with female sex, age at diagnosis less than 75, and epithelioid morphology.[17] A

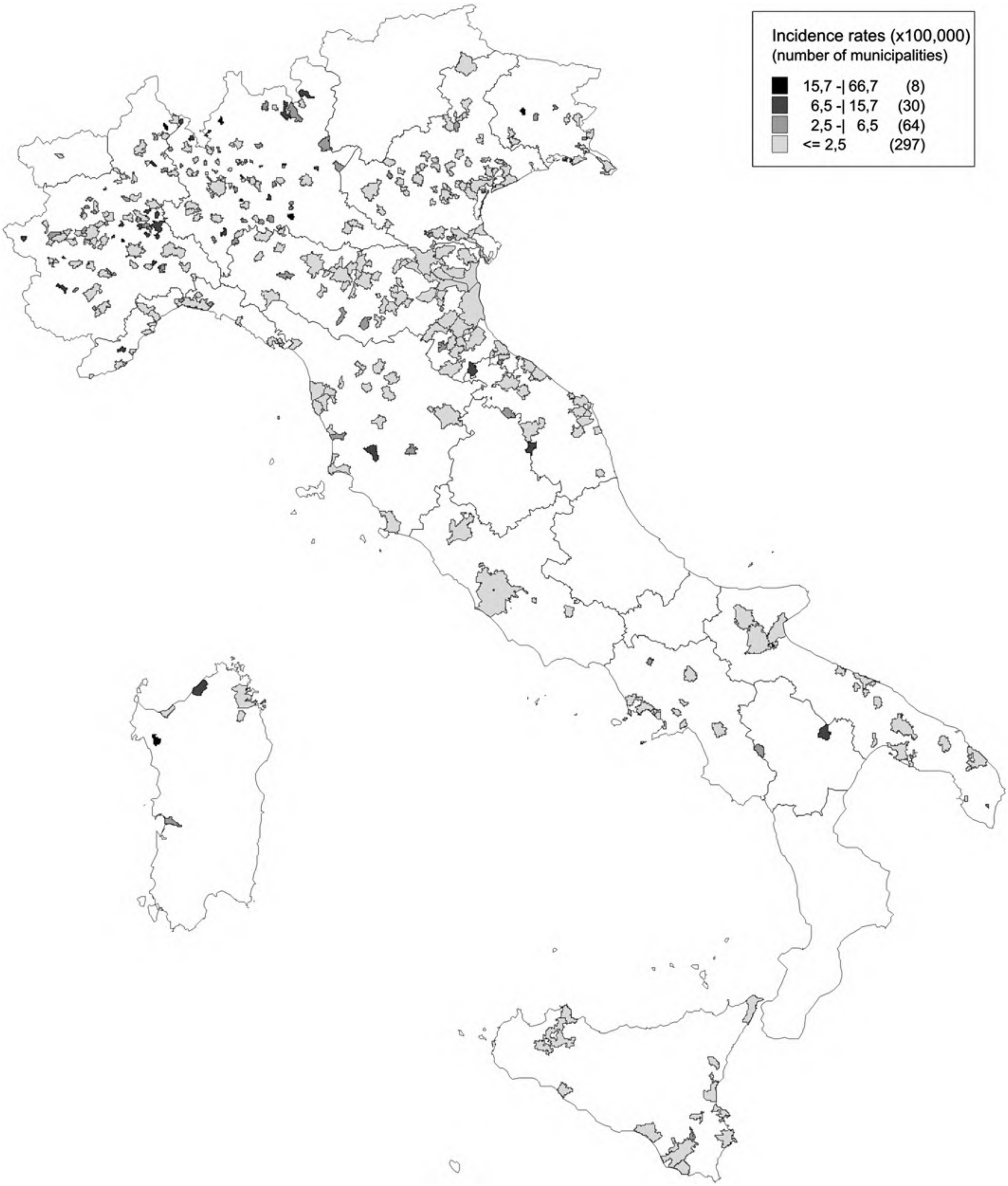

Figure 1 Crude incidence rates of non-pleural cases by Italian municipalities (n=8101). Italian national mesothelioma register (ReNaM). Italy, 1993–2004.

better prognosis for diffuse peritoneal MM has been reported for women, although the reasons are not clear.[18] The many reports of longer survival support the theory of misclassification with ovarian cancer.[12 19]

Epidemiological studies comparing the degree of asbestos exposure with occupation and ultimate site of mesothelioma indicate the peritoneal site as being associated with longer and more intense exposure.[20] Heavy exposures would promote the

EXHIBIT O

Table 2 Distribution of non-pleural malignant mesothelioma (MM) (number of cases and percentage) collected in the Italian national mesothelioma register (ReNaM) by asbestos exposure, Italy, 1993–2004

		Pleura, n (%)			Peritoneum, n (%)			Pericardium, n (%)			T.V. testis, n (%)
		Men	Women	All	Men	Women	All	Men	Women	All	Men
Asbestos exposure*	Occupational	3872 (82.3)	502 (33.6)	4374 (70.5)	188 (75.5)	50 (34)	238 (60.1)	11 (91.7)	1 (11.1)	12 (57.1)	13 (65)
	Household	48 (1.0)	238 (15.9)	286 (4.6)	2 (0.8)	13 (8.8)	15 (3.8)	–	–	–	–
	Environmental	133 (2.8)	161 (10.8)	294 (4.7)	4 (1.6)	13 (8.8)	17 (4.3)	–	1 (11.1)	1 (4.8)	–
	Leisure-related	41 (0.9)	45 (3.0)	86 (1.4)	4 (1.6)	2 (1.4)	6 (1.5)	–	–	–	1 (5)
	Unknown or improbable	613 (13.0)	550 (36.8)	1163 (18.7)	51 (20.5)	69 (46.9)	120 (30.3)	1 (8.3)	7 (77.8)	8 (38.1)	6 (30)
	Partial total	4707 (100)	1496 (100)	6203 (100)	249 (100)	147 (100)	396 (100)	12 (100)	9 (100)	21 (100)	20 (100)
	On-going	1517 (24.4)	765 (33.8)	2282 (26.9)	113 (31.2)	105 (41.7)	218 (35.5)	11 (47.8)	4 (30.8)	15 (41.7)	11 (35.5)
	Total	6224 (100.0)	2261 (100.0)	8485 (100.0)	362 (100.0)	252 (100.0)	614 (100.0)	23 (100.0)	13 (100.0)	36 (100.0)	31 (100.0)

For detailed classification criteria see: Nesti *et al*[11]; Marinaccio *et al*.[14]

migration of fibres to extrapulmonary sites, but it is still not clear how clearance affects this migration to pleural or peritoneal membranes. Bio-persistency of chrysotile fibres in the lung was shorter than for amphiboles.[21] Nevertheless chrysotile fibres have been observed in omentum and/or mesentery of MM cases, with no apparent breakdown, and some were long (≥5.0 μm), suggesting a role in the pathogenesis of peritoneal MM.[22]

The part played by asbestos exposure in the aetiology of peritoneal MM in women and men was investigated in an incident study in Sweden and the Netherlands. During the past 15 years no trend over time was observed in either country, so a role of occupational exposure to asbestos in peritoneal MM should be limited. In Sweden the higher annual incidence rates and the drop observed around 2000 suggested a previous possible misclassification with other peritoneal tumours in women.[8]

Among the few reports on the epidemiology of MM of the tunica vaginalis testis, between 34% and 41% of cases reported a history of asbestos exposure.[23] In the ReNaM case list we found the proportion of testicular MM with ascertained asbestos exposure (70%) was close to that for pleural MM.

The sectors involved in EPMM aetiology confirm the historical importance of asbestos cement industry and shipbuilding in Italy also considering the limited amounts of employed workers. A substantial sex difference in asbestos exposure was observed for pericardial cases (91.7% in men and 11.1% in women) suggesting a difficulty in investigating occupational, residential and familial history, especially for women. This must be taken into account when discussing any hypothetical difference in the attributable fraction for asbestos. The absence of exposures in the shipbuilding, railway and asbestos-cement industries (the sectors 'traditionally' involved in asbestos exposure for pleural MM) for all the 67 pericardial and testicular cases is noteworthy but not easy to interpret and these findings need to be confirmed in a larger sample.

Other risk factors are discussed in the aetiology of pleural mesothelioma such as exposure to different mineral fibres such as erionite or fluoroedenite, ionising radiation and positivity for papovavirus infection.[24–27] Our analysis covering all the Italian provinces provides evidence of a strong, significant correlation between pleural and peritoneal mesothelioma incidence in the general population. This could be due to the high level of territorial disaggregation. Nevertheless, some provinces have a lower peritoneal MM incidence rate than expected, considering the pleural MM incidence, suggesting differences in the capacity for detection of cases. The rarity of EPMM, the low specificity of diagnosis and the problems in identifying the modalities of asbestos exposure limit the possibility of verifying and quantifying the role of these factors. However, the findings of the Italian surveillance system for mesothelioma suggest caution in discussing the role of aetiological factors other than asbestos. The difficulty of clearly identifying occupational exposures in non-industrial work settings, as well as non-occupational

Table 3 Distribution of non-pleural malignant mesothelioma (MM) exposures (number* and percentage) collected in the Italian national mesothelioma register (ReNaM), by anatomical site and economic sector. Industries size as estimated person/years (PY) of observation, Italy, 1993–2004

Economic sector	Peritoneum, n (%)	Pericardium, n (%)	Tunica vaginalis testis, n (%)	Industry size (PY of observation (1000s)
Asbestos-cement industry	58 (21.8)	–	–	30
Building and construction	37 (13.9)	4 (28.6)	3 (18.8)	12540
Metal and steel mechanical industry	26 (9.8)	3 (21.4)	3 (18.8)	11840
Textile	24 (9.0)	1 (7.1)	1 (6.3)	2820
Transport and construction, transport maintenance and repair (no railways or shipbuilding)	17 (6.4)	1 (7.1)	2 (12.5)	5401
Shipbuilding	16 (6.0)	–	–	701
Railway stock	12 (4.5)	–	–	393
National defence	9 (3.4)	–	1 (6.3)	56
Other sectors (all with fewer than eight cases)	67 (25.2)	5 (35.7)	6 (37.5)	NA
All (numbers of exposures)	266 (100.0)	14 (100.0)	16 (100.0)	NA

*Some cases may have had more than one source of exposure.
NA, not available.

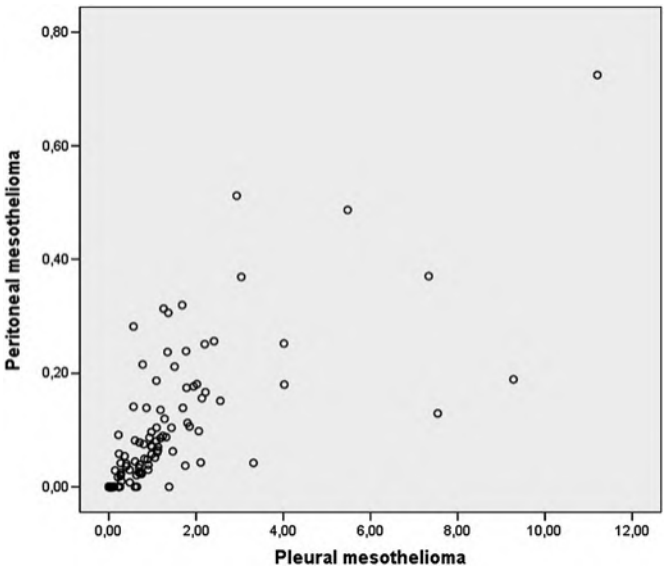

Figure 2 Correlation between pleural and peritoneal mesothelioma raw incidence rates (cases per 100 000 inhabitants) by Italian provinces (N=103). Italian national mesothelioma register (ReNaM). Italy, 1993–2004.

exposure, and the extent of misdiagnosis (which is not easy to quantify) must also be considered in relation to the likelihood of a less close relationship with asbestos for extrapleural MM.

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Incidence of extrapleural malignant mesothelioma and asbestos exposure, from the Italian national register

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Exhibit P

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Short communication

Pericardial mesothelioma and asbestos exposure

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ABSTRACT

Pericardial mesothelioma (PM) accounts for 0.7% of all malignant mesotheliomas. Although asbestos exposure is a recognized etiological factor for pleural and peritoneal mesotheliomas, its role in the development of PM is controversial. The aim of this study is to describe the characteristics of PM cases occurred in Lombardy, a highly industrialized Region of Northern Italy. From the Lombardy Mesothelioma Registry we selected the incident cases of PM registered in the Lombardy Region between 2000 and 2009 and we abstracted clinical characteristics and history of asbestos exposure. We identified 8 cases (6 men and 2 women), with a median age at diagnosis of 55.5 years, representing 0.3% of all mesothelioma cases ($n = 3059$). The age-standardized incidence rate was 0.09 per million/year. Occupational exposure to asbestos was documented in 5 of the 7 cases for which we obtained an interview. Our findings support the role of asbestos in the pathogenesis of PM.

Introduction

Primary pericardial mesotheliomas (PM) are extremely rare, accounting for approximately 0.7% of all malignant mesotheliomas (MM) (Travis et al., 2004). There is a 3:1 predominance of males at all ages. Diagnostic investigations for PM include transthoracic echocardiography, magnetic resonance imaging (MRI), cytology, and biopsy (Permutti et al., 1993).

Only a few case reports have been published and rarely the potential role of asbestos exposure in their genesis has been investigated by collecting information on occupational history (Antman, 1980; Beck et al., 1982; Thomason et al., 1994; Kainuma et al., 2008). However, recently, the International Agency for Research on Cancer (IARC) reaffirmed that all types of asbestos are carcinogenic and may cause MM at any sites (Straif et al., 2009).

The aim of this paper is to describe the characteristics, and in particular the asbestos exposure history, of PM cases recorded between 2000 and 2009 in a population-based registry in Lombardy, a highly industrialized Region of Northern Italy.

Methods

The Lombardy Mesothelioma Registry (LMR), established in 2000, collects all incident cases of MM of the pleura, peritoneum, pericardium, and tunica vaginalis of testis that have been diagnosed in the people residing in the Lombardy region (total population, 9.1 million). Cases are actively reported to the Registry by the main services (pathology, pneumology, surgery, and oncology) of over 100 hospitals in the region. Individual disease history and all available written reports of clinical records (including radiological and histological reports) are collected. Coverage and completeness of LMR are assured by periodic linkage with hospital, local health units, regional, and national databases of: all pathology departments (six-monthly), hospital discharge (yearly), mortality (yearly), and occupational disease records (from the National Institute of Occupational Insurance). Quality indicators were monitored and indicated a good performance: the mortality/incidence ratios were 1.0 and 0.8 for males and females, respectively; moreover, until now there are no cases diagnosed only through a death certificate (so called Death Certificate Only) (Mensi et al., 2007).

Information on asbestos exposure is collected through a standardized questionnaire administered by trained interviewers to the patient or to his/her next of kin. A detailed and complete occupational history comprehensive of industrial sector, patient's job and specific tasks, job of co-workers, and description of workplace is collected. In addition residential history, lifestyle habits, hobbies, and information about job performed by all subjects that lived with the patient are obtained. All records are reviewed and discussed by a panel of experts composed of a pneumologist, an oncologist,

Abbreviations: PM, pericardial mesothelioma; MM, malignant mesothelioma; CT, computer tomography; LMR, lombardy mesothelioma registry; MRI, magnetic resonance imaging; CXR, chest X-ray; PET, positron emission tomography; EC, echocardiography.

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EXHIBIT P

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Table 1
Characteristics of the subject with pericardial mesothelioma, Lombardy region, 2000–2009.

Year of diagnosis	Sex	Age	Survival (days)	Clinical onset	Clinical exams	Histological type	Interview	Job with possible asbestos exposure	Asbestos exposure assessment
2000	M	59	12	Pericarditis	EC, CT, cytology, histology	Epithelial	Next of kin	1956–1975: bricklayer; no other detailed information are available	Occupational/possible: asbestos was widely used in building construction between 1950 and 1980 (Merler et al., 2009)
2001	M	52	408	Serous-hemorrhagic pericardial effusion	CXR, CT, MRI, PET, cytology, histology	Unspecified	NA	NA	NA
2002	M	46	524	Pericardial effusion	CXR, CT, EC, cytology, histology	Epithelial	Patient	1976–2003: firefighter	Occupational/certain: he was exposed both by wearing asbestos clothes and due to fallout from burning buildings
2002	M	77	31	Pericardial effusion	CT, cytology	NA	Patient	1946–1955: metal caster	Occupational/certain: Asbestos was used in the panelling of furnaces and was present in asbestos gloves and other protective devices
2002	F	87	1	Constrictive pericarditis	CXR, EC, histology	Fibrous	Next of kin	1930–1952: shirtmaker in an industrial tailor, using sewing machine for many hours/day. 1956–1980: vegetable farmer, and also bred cattle, swine, poultry, and rabbits; she used recycled jute sacks	Occupational/possible: some brands of sewing machines used during this period employed asbestos brakes, and asbestos materials were also used to insulate some parts of the ironing equipment (Barbieri et al., 2008). Moreover, recycled jute sacks could have previously contained asbestos (Tomasini et al., 1990)
2006	F	45	203	Constrictive pericarditis and pericardial effusion	CXR, EC, MRI, CT, histology	Desmoplastic	Next of kin	1981–1983: postal carrier. 1984–2006: postal clerk	Unknown: although our experience indicates that asbestos was often used in public buildings as a fireproofing material, we could not document the presence of asbestos panelling or insulating materials in the working environment
2007	M	22	1094[a]	Serous pericardial effusion	CXR, EC, CT, MRI, PET, histology	Epithelial	Patient	1998–2000: for a few hours in the summer, as an apprentice mechanic; he denied any asbestos exposure. 2002–2005: bricklayer, but denied handling cement asbestos materials	Unknown: too short time between potential exposure as a bricklayer and disease diagnosis (Consensus Report, 1997)
2009	M	78	0[§]	Pericardial and pleural effusion	CXR; CT; histology	Fibrous	Patient	1946–1984: calenderer in a paper mill	Occupational/certain: the presence of asbestos was documented in insulating pipe and also in the covering of the cylinders of the calandra.

NA = not available; [a]alive at last follow-up; CXR = chest X-ray; CT = computer tomography; MRI = magnetic resonance imaging; PET = positron emission tomography; EC = echocardiography; PM = pericardial mesothelioma; [§]the patient died during surgery.

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a pathologist, an occupational health physician, an epidemiologist, and an industrial hygienist, in accordance with the National Guidelines of the Italian Mesothelioma Registry (Nesti et al., 2003). Further details regarding the LMR methodology have been published elsewhere (Mensi et al., 2007).

For this study, we selected from the LMR database the incident cases of PM recorded between 2000 and 2009 and we abstracted clinical characteristics and history of asbestos exposure.

Results

In the period 2000–2009 we identified 8 cases of PM (6 men and 2 women), which represents 0.3% [95% CI 0.1–0.5] of the total number of mesothelioma cases recorded in the registry during this period (n = 3059 cases of MM of all sites). The age-standardized incidence rates were 0.15 and 0.04 per million/year, respectively for males and females (standard: Italian population, 2001 census).

The details of each PM case are summarized in Table 1. The median (mean) age at diagnosis was 55.5 (58.2) years (range, 22–87), lower than the median age of pleural mesothelioma cases (n = 2897 pleural cases; median age, 68 years; range, 22–91). Clinical onset was pericardial effusion in 6 patients and pericarditis in 3 patients (1 patient had both conditions). Histology was available in 7 cases: 3 MM were epithelial, 3 fibrous, and 1 not specified. The mean (median) survival time was 284 (117) days; one patient survived 1094 days after the date of diagnosis and was alive at the last follow-up (April 8th, 2010).

A standardized questionnaire was completed for 7 cases. For 1 case it was not possible to collect the interview because he had died and his relatives were emigrated at time of interview.

Occupational asbestos exposure was documented in 5 cases (71.4%, 95% CI 29.0–96.3). The median latency (time since first exposure) time was 56 years. In 2 cases, asbestos exposure was classified as unknown because, according to the National Guidelines, "the collected information did not include or exclude asbestos exposure" (Nesti et al., 2003). One case had been working as a postal clerk for many years but was not possible to document the presence of asbestos insulation as a fireproofing in the postal offices where she worked. The other case was a young man in good health: during a routine check-up for a new job, a chest X-ray showed an enlarged cardiac profile. EC and chest CT revealed a significant pericardial effusion with signs of pericardial tamponade. A pericardiotomy confirmed the presence of the epithelial MM. The asbestos exposure questionnaire was administered to the patient. Between 1998 and 2000 (age 13–15) his only employment was, for a few hours in the summer, as an apprentice mechanic; during this period he denied any asbestos exposure. Between 2002 and 2005 (age 17–22) he worked as a bricklayer, but denied handling cement asbestos materials. He did not report any asbestos exposure during leisure time. In view of this history (denial of any asbestos contact), and the fact that, for the job of bricklayer, the latency time (time since start of potential asbestos exposure as a bricklayer) did not comply with the Helsinki Consensus Criteria that require a minimum of 10 years from the first exposure to attribute the MM to that exposure, the asbestos exposure was considered to be unknown.

Discussion

The case-series presented here represented 0.3% of all cases recorded in the LMR during the 2000–2009 period. This result agrees with the National datum of 0.4% in 2004 (Marinaccio et al., 2010) and is similar to the figure of 0.7% of other reports (Travis et al., 2004).

The regional age-standardized incidence rate of PM was 0.09 per million/year. National data for the period 1993–2004 report a total of 36 PM and the incidence rate in the year 2004 was 0.10 per million (Marinaccio et al., 2010).

The mean age of our patients at the time of diagnosis was 58.2 years, in agreement with National data (Marinaccio et al., 2010). On average, PM tended to be diagnosed earlier in life than did pleural mesothelioma.

In the literature, the relationship between asbestos exposure and PM was controversial (Antman, 1980; Beck et al., 1982; Thomason et al., 1994). One reason for this may be that the etiology of PM is rarely investigated, and detailed information on occupational history is seldom gathered (Kainuma et al., 2008; Santos et al., 2008; Vornicu et al., 2007). A recent IARC publication confirmed that asbestos (all types) is a Group 1 carcinogen (carcinogenic to human) and may cause MM of all sites (Straif et al., 2009). Our findings documented a proportion of asbestos exposure of more than 70%, higher than that reported in the literature (Beck et al., 1982; Thomason et al., 1994; Marinaccio et al., 2010) suggesting the importance to obtain detailed occupational history and information on lifestyle habits directly from the patients.

The pericardium involvement is biologically plausible because shares the same embryological origin (mesothelium) of pleura, peritoneum, and tunica vaginalis of testis. It was speculated that asbestos fibers reach the pericardium through a route similar to that of peritoneum, with asbestos directly penetrating the pleura after inhalation and then being transported by retrograde lymphatic flow (Kahn et al., 1980). An alternative hypothesis holds that the asbestos fibers might reach the bloodstream and then be dispersed systemically (Miserocchi et al., 2008).

In conclusion, notwithstanding the rarity PM, we were able to document asbestos exposure in the large majority of case. Our findings support the role of asbestos in the pathogenesis of PM.

Competing interests

All the authors declare that they have no potential competing financial interest.

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Exhibit Q

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Primary Pericardial Mesothelioma: Report of a Patient and Literature Review

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Key Words
Malignant mesothelioma · Pericardium · Review · Asbestos exposure

Abstract
Primary mesothelioma of the pericardium is a rare tumor and carries a dismal prognosis. This case report presents a 38-year-old man who suffered from recurrent pericardial fluid. Initial symptoms were unspecific, with dry cough and progressing fatigue. Pericardiocentesis was performed, but analyses for malignant cells and tuberculosis were negative. After recurrence a pericardiectomy was planned. At operation, partial resection of tumor tissue surrounding the heart was performed. Histopathologic examination including immunohistochemical staining for calretinin showed a biphasic mesothelioma. During the postoperative period the patient's condition ameliorated, but symptoms recurred and the patient died 3 months after diagnosis and 15 months after the first symptoms. At autopsy, the pericardium was transformed by the tumor that also expanded into the mediastinum and had set metastases to the liver. A review of 29 cases presented in the recent literature indicates a higher incidence of malignant pericardial mesothelioma among men than women. Median age was 46 (range, 19–76) years. In pleural mesotheliomas, exposure to asbestos is a known risk factor. However, in primary pericardial mesotheliomas the evidence for asbestos as an etiologic factor seems to be less convincing (3 exposed among 14 cases). Symptoms are often unspecific and cytologic examination of pericardial fluid is seldom conclusive (malignant cells demonstrated in 4/17 cases). Partial resection of the tumor can give a period of symptom reduction. Only a few patients have been treated with chemotherapy. Median survival of patients with pericardial mesotheliomas is approximately 6 months.

Introduction

Malignant diseases of the pericardium can be divided into primary and secondary tumors. Secondary tumors are more frequent, and in this group, metastases from lung or breast cancer are most common [1].

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Malignant mesotheliomas are rare tumors [2] that can occur in any of the body cavities covered by mesothelium. They arise most frequently in pleura but also in peritoneum, pericardium or tunica vaginalis testis. In a large review of 4,710 cases by Hillerdal 1982 [3], pericardial mesotheliomas account for 0.7% of all malignant mesotheliomas. Our report presents one patient with pericardial mesothelioma and a review of 29 cases that appeared in English literature from 1993 through 2008.

Case Report

A 38-year-old metal worker, originally from Cyprus, was admitted to our clinic in October 2002 after over a year's history of recurring pericardial fluid, followed by pericardiectomy and a tumour diagnosis.

In November 2001, he was admitted to a local hospital due to cardiac tamponade. At that time he had a history of several weeks of dry cough and progressing fatigue. Pericardiocentesis with pericardial drain was performed. The fluid was clear and all analyses showed normal results, cultivations and PCR for tuberculosis were negative and cytological analysis of the fluid showed no malignant cells and only a small increase of eosinophilic cells. Chest X-ray was normal except for an increased heart volume; CT scan of the chest/abdomen showed pericardial fluid with a largest width of 4 cm, some amount of pleural fluid and some ascites (fig. 1). The radiological examination was interpreted as exudative pericarditis. A rheumatologist was consulted but could not find any explanation for the patient's condition. Even a small genealogy was made, taking Mediterranean fever into consideration, but with negative results. It finally became clear that the patient had had a bicycle accident 3 weeks prior to the first symptoms from which he had suffered a haematoma in the thoracic wall. Because of this the plausible diagnosis was post-cardiac trauma syndrome. Standard treatment with cortisone was initiated and the symptoms as well as the pericardial fluid disappeared. During the next 6 months the patient had 3 relapses, and in September 2002 he was referred to the University Hospital of Umeå for a pericardiectomy. At surgery, a thickened pericardium was opened and 1 l of filthy and hemorrhagic fluid was exploited. The heart was covered with a 0.5-cm thick layer that gave a constrictive hemodynamic. The layer was to some extent removed, but the area around the phrenic nerve was saved.

The histopathologic examination showed biphasic mesothelioma of the pericardium with positive immunohistochemical staining for calretinin (fig. 2). After surgery, the patient's symptoms diminished and in October 2002 he had recovered. At this time there was no indication for further postoperative treatment. One month later a follow-up CT scan showed pericardial fluid. The patient still had no symptoms. In the beginning of January 2003, his condition worsened with severe dyspnoea and he was admitted to the local hospital. Pleural fluid was stated and he was treated with corticosteroids and diuretics. Ten days later he was transported to our clinic for assessment of further treatment. However, his condition deteriorated and he died 3 months after diagnosis and 15 months after the first symptoms.

Autopsy revealed an advanced tumour that embraced the heart. The pericardium was highly transformed by the neoplasm, thus whitish and solid with a thickness of 3–4 cm in large areas. The myocardium was partly invaded by the tumour and the delimitation between the pericardium and the myocardium was vague. The tumour also expanded from the pericardium to the central parts of the mediastinum. Muddy fluid was noted around the heart. Metastatic tumour growth was found in the liver. A small focus of papillary thyroid cancer with spread to local lymph nodes was also found at autopsy.

Discussion and Literature Review

In 1994, Thomason et al. [4] presented a case report and reviewed 27 cases of primary pericardial mesothelioma that appeared in English literature from 1972 to 1992. After this no extent review has been made. Our review includes 29 cases in English literature from 1993 through 2008 where the diagnosis of primary pericardial mesothelioma was established [5–29]. It also includes the findings from our patient and the review, presented in table 1 and table 2. To provide an overview and comparison, the results from Thomason et al. [4] are presented adjacent to our data.

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Table 1 illustrates that for 30 cases there is a male-female ratio of 3:1. The male domination correlates well with the review by Thomason et al. that reported a male dominance [4]. Andersen et al. [30] reported an even distribution between men and women. On the other hand, they used strict criteria for establishing the diagnosis primary pericardial mesothelioma which excluded cases with distant metastases and is thus not ideal for comparison. The youngest patient in this review was 19 years old and the eldest 76 years old; there is an even distribution of the age range with a median age of 46 years. This corresponds to the review by Thomason et al. in which over half of the patients were diagnosed between the 5th and the 7th decade [4]. An illustration of the age range on the basis of gender shows an even distribution of men and women. No similar illustration is presented in the study by Thomason et al. [4].

No obvious relationship between asbestos exposure and the development of pericardial mesothelioma has been established. Table 1 illustrates asbestos exposure. Three cases with known exposure to asbestos are reported [5–7]. For 11 of the patients no known exposure to asbestos is reported [8–16]. Most of the reviewed articles do not contain any information about asbestos exposure. Thomason et al. reported that a third of the patients were exposed to asbestos [4].

The histological pattern of pericardial mesothelioma is classified into 3 categories according to the World Health Organization: predominantly epithelial, predominantly fibrous (spindle cell) and biphasic (mixed) [31]. Table 2 summarizes the pathologic findings of the patients in this review. The histological pattern was reported in 24 patients and revealed about the same distribution of epithelial and biphasic pattern, 13 and 7 cases, respectively, while the fibrous or sarcomatoid pattern was rarer with only 4 cases. This result is similar to that of the study by Thomason et al., where the distribution was somewhat more even [4]. In the attempt to establish a diagnosis in these patients, cytological study of pericardial effusion is often made. Seventeen cases reported cytological findings from the effusion of pericardial fluid and only 24% of the cases presented malignant cells. The same result was demonstrated by Thomason et al. (20%) and this suggests cytological evaluation to be a poor method for the detection of mesothelioma [4].

The metastatic spread of mesothelioma is presented in table 2. According to Karadzic et al. [17], metastases are present in about 25–45% of the patients and involve regional lymph nodes, lungs and kidneys. Eren et al. [32] report a similar incidence of metastatic spread. In our study, 16 of the case reports contained information about metastatic spread, and 6 of them had no metastatic spread, 8 had regional lymph node involvement, 2 had liver metastases and 1 patient had lung metastases.

Survival distribution of the patients from the time when the first symptoms appeared is illustrated in **fig. 3**. Three patients were alive when the article was published [18], and for 2 patients no information on survival was presented [19]. The median survival time from first symptoms in this review is 6 months. This dismal prognosis correlates with earlier studies; Cohen [31] showed average disease duration of approximately 5 months. No similar diagram was presented in the study by Thomason et al. [4].

In summary, this review of the clinical characteristics of patients with primary pericardial mesothelioma shows that initial symptoms are unspecific. Whether exposure to asbestos is related to the disease or not is uncertain. Treatment options are limited and the prognosis is dismal.

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Table 1. Clinical material, gender, age and asbestos exposure

	n	%	Thomason et al. [4], %
Gender			
Men	22	73	71
Women	8	27	29
Exposure to asbestos			
Exposure	3/14	21	33
No known exposure	11/14	79	67
Not mentioned	16/30	53	57
	n	**Median (min.–max.)**	**Thomason et al. [4]**
Age, years			
Men	22	45.5 (19–70)	
Women	8	52.5 (31–76)	
All	30	46 (19–76)	48 (12–77)

Table 2. Histopathologic and metastatic findings

	n	%	Thomason et al. [4], %
Mesothelioma type			
Biphasic	7/24	29	35
Epithelial	13/24	54	35
Sarcomatous	4/24	17	30
Not available	6/30	20	28
Effusion cytologic findings			
Benign	10/17	59	70
Malignant	4/17	23	20
No fluid available	3/17	18	–
Inconclusive	–	–	10
Not available	13/30	43	64
Metastases			
Lymphatic	8/16	50	
Hepatic	2/16	13	
None observed	6/16	38	
Not mentioned	14/30	47	

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Fig. 1. CT-scan of the thorax of the 38-year-old patient at presentation of symptoms.

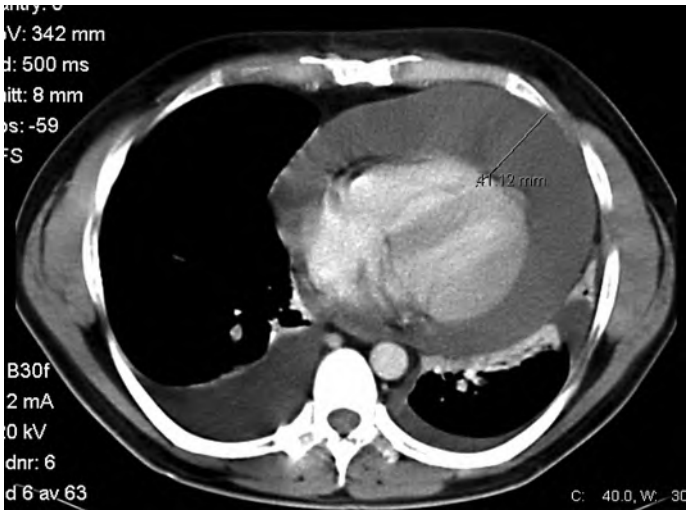

Fig. 2. Immunohistochemical staining for calretinin (Zymed®, polyclonal rabbit anti-calretinin antibody, Invitrogen, Carlsbad, Calif., USA) showing a biphasic mesothelioma (20X).

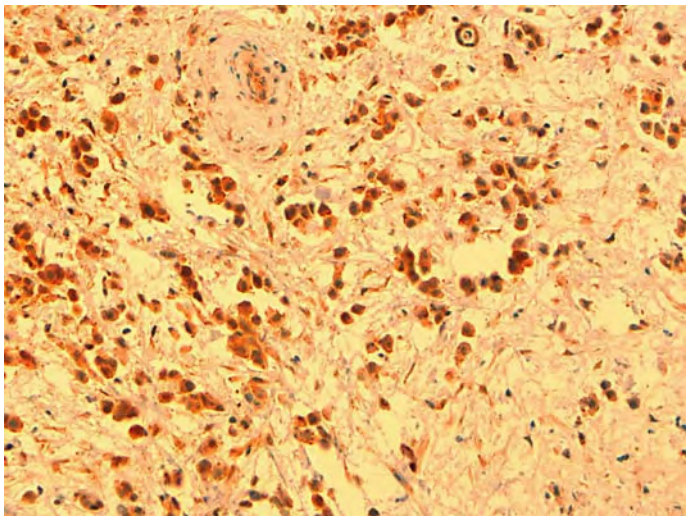

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Fig. 3. Kaplan-Meier plot of disease-specific survival of 30 patients with pericardial mesothelioma [5–29].

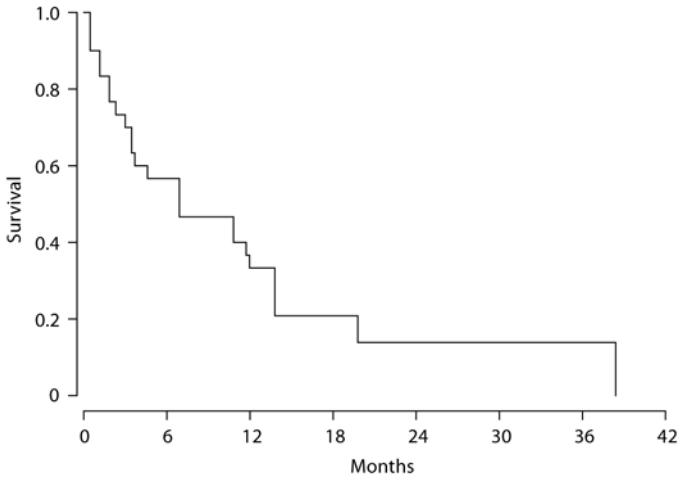

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Exhibit R

Tumori, *91*: 276-279, 2005

MALIGNANT PERICARDIAL MESOTHELIOMA. REPORT OF TWO CASES, REVIEW OF THE LITERATURE AND DIFFERENTIAL DIAGNOSIS

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Malignant pericardial mesothelioma is an uncommon variety of a primary malignant cardio-pericardial tumor and it is a highly lethal and fortunately rare cardiac neoplasm. The presentation of pericardial mesothelioma is aspecific and pathologically mesothelioma is not the most common among primary tumors of the pericardium. It is characterized by atypical solid growth of mesothelium with formation of atypical cavities surrounded by fibrous stroma. Antemortem diagnosis is difficult and distant metastases are extremely rare. Radical surgery can be used to treat localized mesothelioma. The treatment for advanced primary pericardial mesothelioma is usually palliative because the tumor is resistant to radiotherapy and chemotherapy. The prognosis is unfavorable. The median survival from the onset of symptoms is six months. In this paper we report two cases of patients with primary mesothelioma of the pericardium without a definite history of asbestos exposure.

Key words: asbestos exposure, malignant pericardial tumors, mesothelioma.

Introduction

Mesothelioma is a malignancy deriving from the serous epithelial cells of the mesothelium; the most frequent sites are the pleura (60-70%) and the peritoneum (30-35%); mesothelioma of the pericardium or the vaginal epithelium of the testicle is very rare[1]. A study of about 500,000 autopsies revealed a pericardial mesothelioma incidence lower than 0.0022%, or about 2-3% if we consider only autopsies of patients who died of cancer[2]. Mirabella[3] carried out an epidemiological study of 206 cases of pericardial mesothelioma from 1875 to 1978.

The aim of this paper is to report two cases of pericardial mesothelioma with emphasis on some aspects of their clinicopathological presentation that complicated the diagnosis.

Case 1: Clinical presentation

A 68-year-old retired man, ex smoker, came to our attention in October 1997. His history reported surgery for a right inguinal hernia at approximately 50 years of age and a gallbladder resection when the patient was in his sixties.

In May 1997 the patient complained of dyspnea, asthenia, evening fever and weight loss. Because of a general decline in his overall condition he was admitted to hospital, where a chest X-ray revealed an enlargement of the cardiac shadow due to massive pericardial effusion and moderate right pleural effusion. About 1000 mL of hemorrhagic effusion was obtained through pericardial drainage. Cytological analysis revealed the presence of epithelial tumor cells of unknown origin. The patient underwent a bronchoscopy, colonoscopy, abdominal ultrasonography and thoracic CT scan. Although these exams did not reveal the presence of malignancy, chemotherapy with cisplatin (80 mg/m^2 day 1) and navelbine (30 mg/m^2 day 1-8) every 28 days was started. After one month the patient was hospitalized following an inferior acute myocardial infarct. Echocardiogram and chest X-ray revealed pericardial effusion, hence the patient underwent pericardial resection and right ventricle suture. Histological examination demonstrated that the patient suffered from a pericardial mesothelioma. He returned to our department for the prescribed chemotherapy, but because of his worsening general condition it was decided to follow a palliative care protocol. Another chest X-ray confirmed the presence of bilateral pleural and pericardial effusion. About 1800 mL of serous effusion was collected through thoracentesis. The general condition of the patient worsened very rapidly and he died after admission to the Internal Medicine Department.

Case 2: Clinical presentation

A 44-year-old male painter, a current smoker (about 30 cigarettes/day), came to our attention in 1998. His history reported a tonsillectomy at age 12. In October 1998 the patient had begun wide-spectrum antibiotic treatment for a tooth abscess. After seven days of treat-

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EXHIBIT R

ment he manifested general malaise, chills, fever, asthenia, chest and joint pain that did not respond to anti-inflammatory drugs. The patient was hospitalized in the infectious disease ward, where he underwent a chest X-ray to exclude the presence of pneumonia. This only revealed pericardial effusion, so the patient was transferred to the cardiological intensive care unit where he underwent pericardial drainage which collected about 1100 mL of hemorrhagic effusion. Cytological analysis did not reveal atypical cells.

After one week it was necessary to perform a sternotomy with pericardial drainage and biopsy sampling because the patient manifested symptoms of cardiac tamponade. Histological examination revealed pericardial mesothelioma. Following surgery, given the worsening of his general condition, the patient was transferred to the Intensive Care Unit where he later died.

Histopathological description

Case 1: Pathological findings (Figure 1)

Four biopsy samples of white-yellow fibrous tissue were analyzed, the largest measuring 8 × 4 × 2 cm. Microscopic examination revealed cancer infiltration of the pericardium with a multiple connected nodule morphology. These nodules consisted of large epithelial elements with well-marked vacuolated eosinophil cytoplasm, a vesicular nucleus and prominent nucleolus (Figure 1b). The cells formed cord-like or tubular-papillary structures, confirming the mesothelial origin of the malignancy. Foci of epithelial mesothelioma *in situ* were present (Figure 1a). Foci of atypical hyperplastic mesothelium were present in areas not affected by the malignancy.

Immunohistochemistry was positive for cytokeratin (AE1/AE3) (Figure 1c) and calretinin (Figure 1d); there was diffuse membrane positivity for EMA and HBME-1 and focal positivity for vimentin, while staining for CEA and CD15 was negative. The diagnosis was pericardial epithelial mesothelioma.

Case 2: Pathological findings (Figure 2)

Several hard pericardial samples with irregular shapes were analyzed, the largest measuring 7 × 4.5 × 2 cm. Histological examination revealed a pericardium affected by a malignancy lacking differentiation with

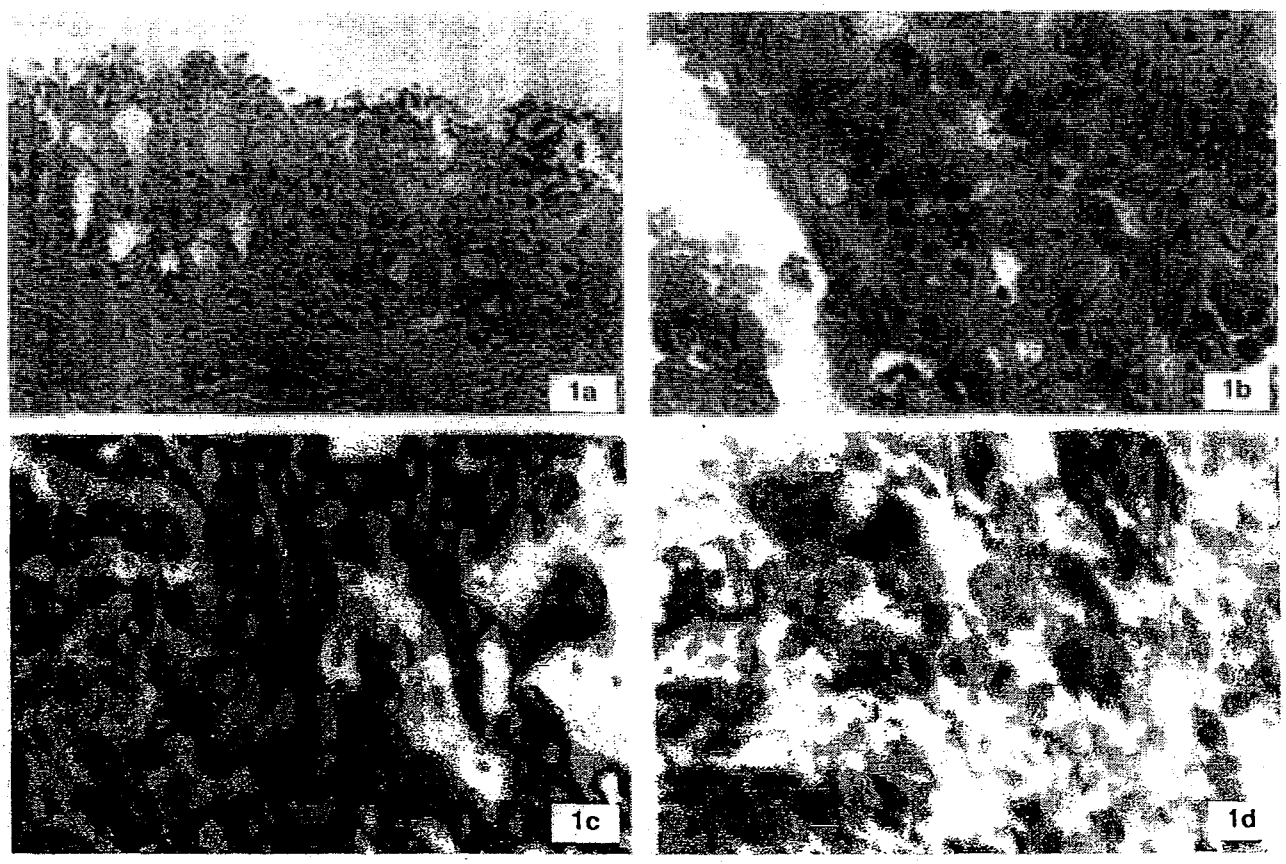

Figure 1 - Case 1: 1a) Epithelial mesothelioma *in situ* (EE). 1b) Atypical epithelial mesothelioma with tubular-papillary growth. 1c) Marked presence of cytokeratin (AE1, AE3). 1d) Many elements show calretinin.

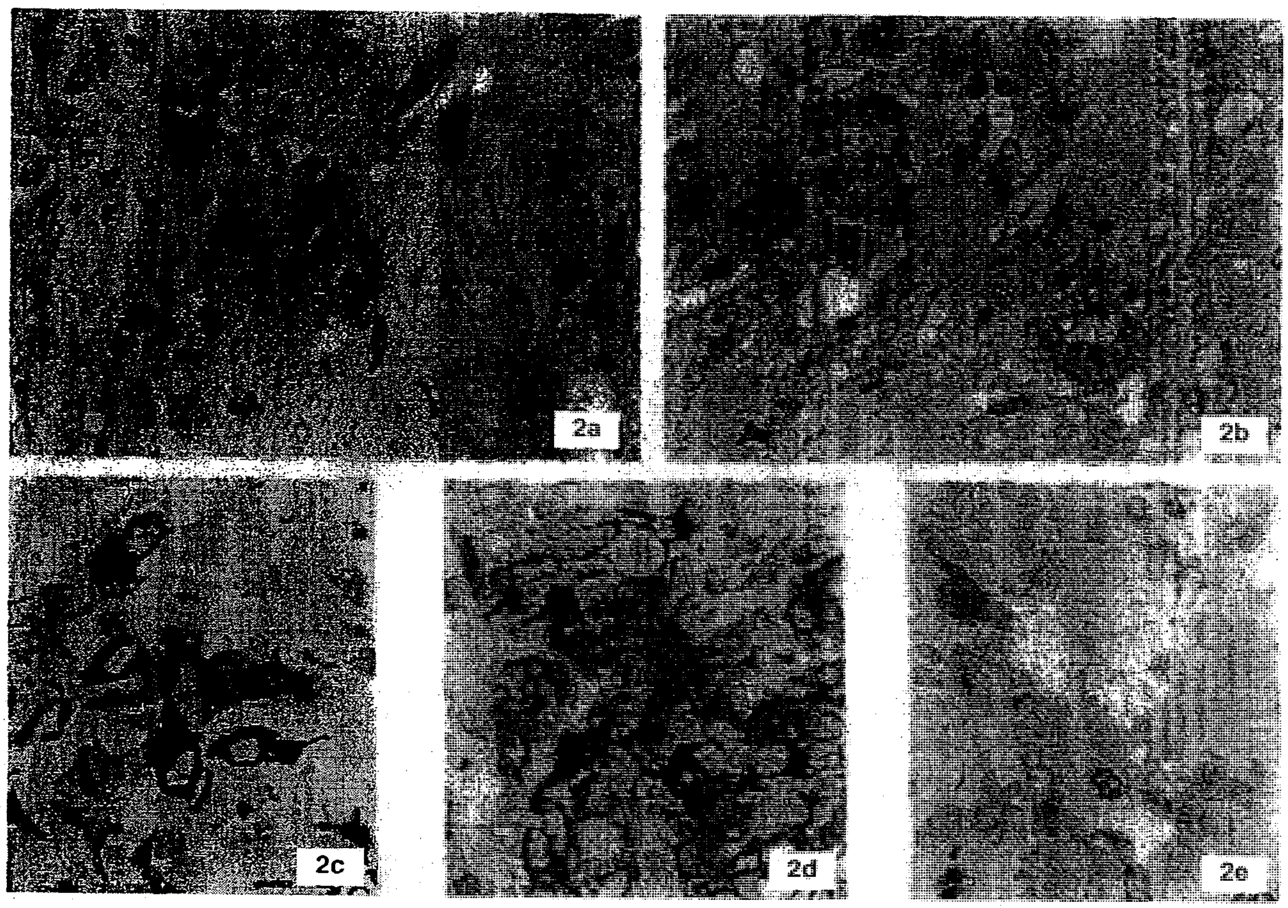

Figure 2 - Case 2: 2a-b) Mesothelioma with sarcomatoid appearance and areas with angiosarcoma-like differentiation. 2c) Diffusely positive for AE1/AE3. 2d) Diffusely positive for vimentin. 2e) Focally positive for calretinin.

confluent nodules and plaques. The cancer cells had an extended shape with a large nucleus, prominent nucleolus and vacuolated cytoplasm, and there was angioblastic differentiation (Figure 2a-2b); the neoplastic cells rarely had a extended shape and formed cord-like structures. The malignancy was positive for AE1/AE3 (Figure 2c), vimentin (Figure 2d), calretinin (Figure 2e), EMA and HMB-1, and negative for CEA, CD15, CD34 and Factor VIII. The diagnosis was biphasic mesothelioma, sarcomatous in the pericardium: this diagnosis was confirmed by the autopsy findings (Figure 3).

Discussion

The clinical features of pericardial effusion are chest pain, dyspnea, cough and sometimes paradoxical pulse. Pericardial effusion can be detected by chest X-ray, echocardiography and MRI or CT scan of the chest. The most frequent cause of effusion is *Mycobacterium tuberculosis* infection. Other frequent causes are myxedema, systemic lupus erythematosus, rheumatoid arthritis, severe chronic anemia, chylopericardium, mycetes/pyogenic infection, radiotherapy, and hypercholesterolemia. The presence of hemorrhagic effusion may indicate an infection (eg tuberculosis) but also chronic hemorrhage due to an aortic aneurysm must be taken into account.

Hemorrhagic pericardial effusion could also be pro-

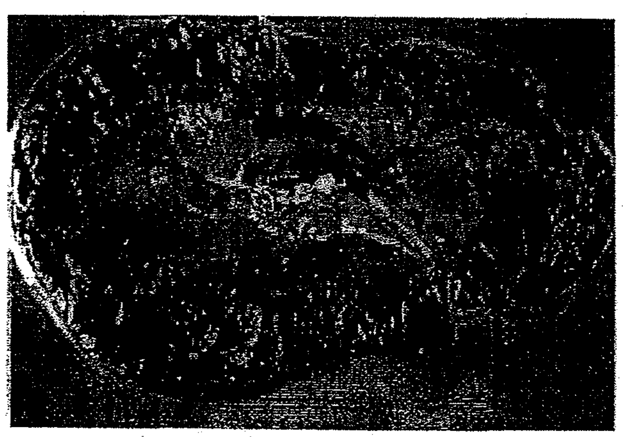

Figure 3 - Case 2: autopsy report.

duced by a distant malignancy that metastasized to the pericardial serosa; this process accounts for about 7% of all hemorrhagic pericardial effusion[4]. Also the heart could be affected by tumors that subsequently produce pericardial effusion. Autopsy studies found the heart to be affected by metastasis in 0.25% to 6.5% of all malignancies, with lung cancer being the prevailing primary site[5]. Primary heart malignancy accounts for 0.0017% to 0.28%, as was found on the basis of random autopsies (eight primary malignancies in a total of 480,000 autopsies performed in the US from 1938 to 1942)[6].

Primary heart mesothelioma accounts for about 2-3% of all cardiac and pericardial primary tumors and about 1% of all mesotheliomas[7]: it is the third tumor after angiosarcoma (33%) and rabdomyosarcoma (20%)[8]. Exposure to asbestos is correlated with the onset of pleural and peritoneal mesothelioma; however, the role of asbestos in pericardial mesothelioma is unclear.

The diagnosis is made on the basis of cytological examination, ultrasound or CT-guided biopsy, and MRI[9]; in only 10-20% of cases can a diagnosis be made before the death of the patient. It is important to differentiate between malignancy and mesothelial reactive hyperplasia associated with inflammatory disease[10]. Features that indicate the presence of a malignancy are infiltration of deep tissues, atypical cytoplasm, necrosis and confluent forms. If deep tissue infiltration is not present, the diagnosis can be based on severely atypical cytoplasm. Immunohistochemistry is useful for the differential diagnosis, but it is necessary to obtain additional information (anamnestic, clinical or radiological). Mesothelioma cells stain positive for cytokeratin, vimentin, epithelial membrane antigen (EMA) and calretinin, and negative for CEA, CD15 and S-100[11,12]. Pericardial mesothelioma infiltrates the myocardial and mediastinal structures. Metastases are present in about 25-45% of the cases and involve the regional lymph nodes, lungs and kidneys[13].

As far as treatment is concerned, several studies have shown the efficacy of surgery, radiotherapy and chemotherapy, but the results are modest and provide no significant difference in prognosis, which remains poor (the median survival is about six months from diagnosis)[14]. Sometimes the malignancy involves the atria-ventricular nodes with a third-degree conduction block or the coronary circulation with ischemic heart attack[15,16]. The most frequent causes of death are cardiac tamponade, vena cava occlusion and congestive heart failure.

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Exhibit S

ANTICANCER RESEARCH 30: 1323-1326 (2010)

Primary Pericardial Mesothelioma in an Asbestos-exposed Patient with Previous Heart Surgery

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Abstract. *We present a case of primary pericardial mesothelioma occurring in an asbestos-exposed 67-year-old man who underwent four aortocoronary bypass grafting seven years prior to the onset of the mesothelioma. Primary pericardial mesothelioma is a rare tumor whose association with asbestos is more infrequent than that of the much more common pleural form. Factors other than asbestos that may play a role include genetic predisposition, immune impairment, infections, radiation, dietary factors, and recurrent serosal inflammation. We consider that, in the presented case, inflammation and healing resulting from pericardiotomy might have had a synergistic effect with asbestos in the pathogenesis of the tumor. To our knowledge, this is the first reported case of primary pericardial mesothelioma arising in a patient exposed to asbestos who previously underwent cardiac surgery.*

Primary pericardial mesothelioma is a rare tumor whose association with asbestos is more uncommon than that of the much more frequent pleural form. Factors other than asbestos that may play a role include genetic predisposition, immune impairment, infections, radiation, dietary factors, and recurrent serosal inflammation. We present a case of primary pericardial mesothelioma occurring in an asbestos-exposed patient following cardiac surgery, in which inflammation and repair subsequent to pericardiotomy might have had a synergistic effect with asbestos in the pathogenesis of the tumor.

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Key Words: Pericardial mesothelioma, mesothelioma, asbestos, inflammation.

Case Report

In July 2008, a 67-year-old former plumber was admitted to the Cardiology Department for persistent chronic heart failure with increasing dyspnea and orthopnea. His clinical history was significant for an acute myocardial infarction involving both left and right ventricular walls which had occurred seven years before and had been treated with four aortocoronary bypass grafts. Physical examination revealed crepitant rales over 50% of lung fields, tachyarrhythmia, and dependent edema. Laboratory findings showed renal failure (creatinemia 1.9 mg/dl, uremia 110 mg/dl), increased C-reactive protein, erythrocyte sedimentation rate and white blood cell count, and slight elevation of CA 15.3 (28.1 U/ml, n.v. 0.25 U/ml); however, normal values of other tumor markers such as α-fetoprotein, carcinoembryonic antigen (CEA), and cancer antigen (CA) 19-9 were confirmed. Chest x-ray demonstrated bilateral pleural effusion, widespread interstitial involvement with reticular nodulation, and cardiomegaly. Thoracic CT scan demonstrated prominent pericardial effusion, irregular calcified thickenings of the pleura, partial collapse of the lower lung fields, and multiple parenchymal opacities associated with interstitial involvement with reticular nodulation (Figure 1). Echocardiography and transesophageal echography showed severe biventricular dysfunction, apical right ventricular thrombosis, and periapical and posterior pericardial thickening that was interpreted as pericardial effusion. A thoracentesis removed 500 ml of straw-colored fluid in which cytological examination revealed the presence of atypical mesothelial cells, occasionally clustered in papillary structures, highly suggestive of mesothelioma. The patient was transferred to the Department of Internal Medicine, where he underwent an additional thoracentesis, right thoracic drainage, and thoracoscopic talc poudrage. The patient self-discharged with the diagnosis of probable pleural mesothelioma, ischemic cardiopathy, heart failure NYHA class IV with severe biventricular systolic dysfunction, left ventricular thrombosis, and pericardial effusion. He subsequently died 40 days later.

EXHIBIT S

ANTICANCER RESEARCH *30*: 1323-1326 (2010)

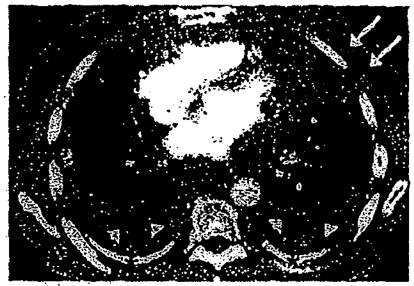

Figure 1. *Axial CT scan of the chest showing prominent pericardial effusion (arrows). Calcified pleural plaques (asterisks) and pleural effusion (arrowheads) are also evident.*

Figure 2. *Microphotograph showing malignant mesothelioma of epithelioid type originating from the epicardium and infiltrating the myocardium.*

At post-mortem examination, a lardaceous sleeve reaching 2 cm in maximum thickness at the ventricular apex encircled the right heart and infiltrated the pericardium with adherence of the heart to the posterior surface of the sternum. Post-pericardiotomy adhesions and diffuse epicardial sclerosis were also evident. Native coronary arteries were diffusely calcified and stenotic, and the anterior descending branch of the left coronary artery was completely obliterated, while the aortocoronary bypass grafts were patent. A wide posterior endomyocardial scar corresponding to the previous infarction was observed. There was conspicuous accumulation of fluid in the pleural space, and multiple diaphragmatic and parietal pleural plaques, some calcified, with the largest measuring about 9 cm. The visceral pleural surface was disseminated with numerous small neoplastic nodules that were also present in the pulmonary parenchyma. The quantification of asbestos bodies in the lung parenchyma, performed in accordance with the method of Smith and Naylor with slight modifications, revealed the presence of 1800 asbestos bodies per gram of dry lung tissue, consistent with an occupational exposure to asbestos. The histological picture was that of an epithelioid mesothelioma arising from the epicardium with deep invasion of the myocardium (Figure 2) and parietal infiltration of the coronary veins, and metastatic dissemination extensively involving the visceral pleura and the lung parenchyma. Immunohistochemically, the neoplastic cells were positive for cytokeratins 5/6, epithelial membrane antigen (cytoplasmic positivity with membrane enhancement), calretinin, weakly positive for vimentin, and negative for CEA and TTF-1.

Discussion

Mesothelioma of the pericardium is a rare tumor: fewer than 150 cases have been reported in the literature. It accounts for 0.7% of all malignant mesotheliomas, the majority of which originate from the pleural lining. The mean age of patients at presentation of pericardial mesothelioma is 46 years, with an age range of 2 to 78 years. The male to female ratio is nearly 2 to 1. The majority of patients with pericardial mesothelioma present with dyspnea, and cardiomegaly caused by pericardial effusion or solid tumor infiltration. Cardiac tamponade often develops during the course of the disease. Although effusion is the rule, the pericardial cavity may be obliterated by tumor, explaining the lack of fluid at pericardiocentesis in some cases. Echocardiography, CT and MR are the main diagnostic imaging techniques used. Echocardiography is generally effective in distinguishing solid tumor infiltration of the pericardium from effusion. Definitive diagnosis is based on cytologic examination of pericardial fluid, supported by the evaluation of biopsy samples which normally demonstrate typical histologic and immunohistochemical features (1, 2). Andersen and Hamsen's criteria to identify a primary pericardial mesothelioma require that there is no tumor present outside the pericardium with the exception of lymph node metastasis (3). With such certainty that such criteria are excessively restrictive, we concluded that the tumor was a pericardial primary on the basis of the extent of involvement of the pericardium compared to the pleura.

The association between malignant mesothelioma of the pleura and asbestos exposure is well known. It is currently believed that, like pleural mesotheliomas, at least some mesotheliomas of the pericardium are caused by asbestos (1). A case of pericardial mesothelioma that developed 15 years after pericardial dusting with asbestos and fiber glass as a treatment for angina pectoris has been described (4). The patient described in the present report lived in Monfalcone, a ship-building town in north-eastern Italy with a high incidence of mesothelioma. He had a history of asbestos exposure, confirmed by quantification of asbestos bodies in his lung parenchyma, indicating an occupational level of exposure. Nevertheless, the link with asbestos is weaker for

pericardial than for pleural mesothelioma. The proportion of women with pericardial mesothelioma is higher than that with pleural mesothelioma. In most cases of reported pericardial mesothelioma, no history of asbestos exposure is mentioned (1). Furthermore, cases of pericardial mesothelioma due to nonasbestos-related causes have been reported, including that of radiotherapy (5, 6).

An increasing body of evidence indicates that factors other than asbestos play a role in the pathogenesis of malignant mesothelioma, including genetic predisposition, immune impairment, infections, radiation, and dietary factors. Recurrent serosal inflammation has also been reported to represent a possible condition predisposing to malignant evolution. Mesothelioma generally develops on pleura affected by pleural plaques, which are the effect of recurrent inflammatory and repair processes occurring over decades (7). Moreover, cases of pleural mesothelioma secondary to chronic inflammation and old scars, observed after chronic empyema or therapeutic pneumothorax, have been described (8-10). The link between inflammation and cancer was first noticed by Virchow in 1863. Since this early observation, accumulating studies have supported that chronic inflammatory diseases are frequently associated with an increased risk of cancer. In a setting of chronic inflammation, the persistent tissue damage and cell proliferation as well as the enhanced production of reactive oxygen and nitrogen species contribute to a cancer-prone microenvironment. A variety of mediators, including cytokines, chemokines, and enzymes, may also facilitate cancer development via multiple signaling pathways (11).

In this case, inflammation and normal repair following pericardiotomy might have had a synergistic effect with asbestos in the pathogenesis of the tumor. To our knowledge, this is the first reported case of primary pericardial mesothelioma arising in a patient exposed to asbestos and previously subjected to cardiac surgery.

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Exhibit T

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Primary Malignant Pericardial Mesothelioma Presenting as Effusive-Constrictive Pericarditis

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Primary Malignant Pericardial Mesothelioma Presenting as Effusive-Constrictive Pericarditis

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Section:
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Issue Number: Volume 23 - Issue 8 - August 2011 [2]
Author(s):

Parikshit S. Sharma, MD, MPH, and Dennis Katechis, DO

ABSTRACT: Effusive-constrictive pericarditis is a clinical hemodynamic syndrome characterized by constriction of the heart by the visceral pericardium in the presence of a tense pericardial effusion. The hallmark of effusive-constrictive pericarditis is the persistence of elevated right atrial pressures and ventricular interdependence after relief of the elevated intrapericardial pressures. The present report discusses the unique case of a 46-year-old white female who presented with dyspnea on exertion and chest tightness in the setting of an effusive-constrictive pericarditis. The patient was subsequently diagnosed with primary malignant pericardial mesothelioma, an extremely rare neoplasm with a very poor prognosis.

J INVASIVE CARDIOL 2011;23:E197–E199

Key words: pericarditis, effusive-constrictive pericarditis, pericardial mesothelioma

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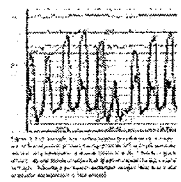
Case Report. *This case presents a 46-year-old white female, a former smoker with a past medical history of non-insulin dependent diabetes, polycystic ovarian syndrome and depression. She is a retired insurance agent with a remote history of occupational exposure to asbestos. The patient was seen by her primary care physician and sent to the emergency room at an outside hospital with a 3-day history of dyspnea on exertion and chest tightness. The dyspnea worsened*

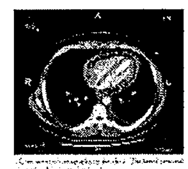
EXHIBIT T

on minimal activity and was progressive in nature. Chest tightness was midsternal in location, without radiation, and worsened with deep inspiration.

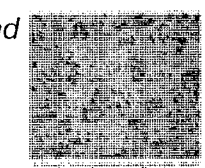
On triage, the patient was hypertensive with a blood pressure of 141/107 mmHg, tachycardiac with a pulse rate of 107 bpm. Physical exam was remarkable only for distant heart sounds without any jugular venous distension. Laboratory analysis revealed a troponin T of 0.06 ng/ml, with a normal CPK and CK-MB. Electrocardiogram done in the emergency room showed sinus tachycardia with a ventricular rate of 104 bpm and low voltage complexes. Chest x-ray demonstrated cardiomegaly and pulmonary vascular congestion. A computerized tomography (CT) scan of the chest ruled out pulmonary embolism and aortic dissection, but found a large pericardial effusion. A transthoracic echocardiogram (TTE) confirmed presence of a moderate-sized pericardial effusion with right artial collapse in systole and significant respiratory variation of the mitral valve inflow pattern.



[3]Given the TTE findings, which were suggestive of early tamponade physiology, the patient underwent an emergent pericardial window via subxiphoid approach on day 1 of hospitalization. A follow-up postoperative TTE showed presence of constrictive physiology as suggested by paradoxical septal wall motion and respiratory variation of the mitral inflow and cardiac output. The patient underwent a simultaneous right and left heart catheterization, which demonstrated right artial pressure of 21 mmHg, right ventricular pressures of 55/22 mmHg, a pulmonary artery systolic pressure of 56 mmHg, with left ventricular end diastolic pressure of 22 mmHg and prominent X and Y descent of the right atrial pressure tracing, marked respiratory variation in pressures and equalization of chamber pressures during diastole, and the dip-plateau morphology of left intraventricular pressure (Figure 1).



[4]A repeat CT scan of the chest done on day 6 revealed a moderate pericardial effusion with nodularity of the pericardium (Figure 2), without evidence of any pleural lesions. The CT scan findings were suggestive of a pericardial neoplasm and the patient underwent a pericardiectomy on the beating heart without cardiopulmonary bypass. In the operating room, the patient was found to have a thickened parietal pericardium with a small-sized pericardial effusion and a thick, adherent visceral pericardium.



[5]Pathology reports revealed vesicular nuclei with prominent nucleoli (Figure 3) and immunohistochemical staining confirming mesothelial origin of the tumor. The patient gradually recovered and was eventually discharged with plans for prompt initiation of outpatient chemotherapy.

Discussion. Effusive-constrictive pericarditis is an uncommon pericardial syndrome that was first described in the 1960s. In a prospective study of 1,184 patients with pericarditis, Sagrista-Sauldea et al reported that 6.9% of 218 patients with tamponade had confirmed effusive-constrictive pericarditis.[1] The most common cause of effusive-constrictive pericarditis is idiopathic, while cases can be secondary to irradiation, cardiac surgery, uremia, malignancy, or infections like tuberculosis.

Our patient was initially found to have a pericardial effusion with early tamponade physiology. The pressure tracings from a right and left heart catheterization done after relieving the effusion unveiled equalization of diastolic pressures in the 4 cardiac chambers, which was more prominent during inspiration (Figure 1). As noted in Figure 1, there was also evidence of discordance of pressure changes with respiration in the 2 ventricles, a phenomenon called ventricular interdependence, and the presence of a dip and plateau configuration of left ventricular tracings (square root sign) following a premature ventricular complex. All of the above hemodynamic findings are characteristic of a constrictive physiology and with the presence of the

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PMPM is an extremely rare neoplasm with a reported incidence of < 0.0022% in a large necropsy series.[2] However, it is also the most common primary pericardial tumor. There is a possible association with asbestos exposure, but this is seen only in cases with coexistent asbestos-related pleural disease.[3] The onset of symptoms is insidious, with presentation usually occurring late in the course of the disease. Common presentations include constrictive pericarditis,[4] cardiac tamponade,[5] and heart failure. Other complications such as distant metastasis, heart block,[6] and tumor embolism causing neurological deficits[7] have also been reported. Upon review of the literature, effusive-constrictive pericarditis as a complication of PMPM has never been reported. CT, magnetic resonance imaging, and two-dimensional echocardiography are useful in identifying the tumor and its extent. Histology and immunohistochemistry with positive staining for calretinin, cytokeratin 5/6, and mesothelin of the biopsy sample are necessary to confirm the diagnosis.

The overall prognosis of PMPM is very poor.[8] The response to radiation is poor. Cyclical combination chemotherapy with doxorubicin, vincristine, and cyclophosphamide may reduce the tumor mass.[9] Surgical interventions such as pericardiectomy may help cure local tumors, or provide palliative care by decreasing disease burden and offering symptom relief.

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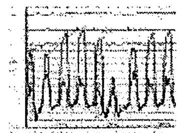

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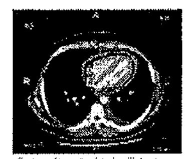

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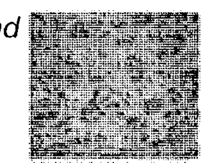

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Exhibit U

Case Reports

Primary Malignant Mesothelioma of the Pericardium

Case Report and Literature Review

Ronald Thomason, MD
William Schlegel, DO
Michael Lucca, MD
Steven Cummings, MD
Scott Lee, MD

Pericardial mesothelioma is a highly lethal and fortunately rare cardiac neoplasm. We present the clinical and pathologic features of a primary sarcomatoid mesothelioma. To better understand the clinical, radiographic, and pathologic features of this entity, we reviewed 27 cases described in the English literature from 1972 through 1992, which, together with our case, provided a total of 28 cases. Findings of the review include a male-female ratio of 2:1, a wide age range (12 to 77 years; mean, 47 years), and documented asbestos exposure in 4 of 28 (14%) patients. Commonly used imaging studies do not appear to offer great sensitivity, for a mass was detected by echocardiography in only 2 of 16 (12%) patients and by computed tomography in 4 of 9 (44%). Pathologic findings revealed a diffuse growth pattern in most cases (18 of 25, or 72%), together with an equal distribution between the biphasic, epithelioid, and sarcomatoid variants. Effusion cytology revealed malignant cells in only 2 of 10 (20%) cases. With or without therapy, prognosis was uniformly poor, since 24 of 27 patients were dead of the disease at the time the reports were published. ***(Texas Heart Institute Journal 1994;21: 170-4)***

Key words: *Asbestos; cardiac tamponade; heart neoplasms; heart surgery; immunohistochemistry; mesothelioma/pathology; myocardial infarction; pericardial effusion; pericardium*

From: *The Departments of Cardiology (Drs. Schlegel and Lucca), Pathology (Drs. Thomason and Lee), and Cardiothoracic Surgery (Dr. Cummings), Wilford Hall Medical Center, Lackland Air Force Base, Texas 78236-5300*

The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Defense or other Departments of the U.S. Government.

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Pericardial mesothelioma is a rare and highly lethal cardiac tumor. There has been no organized review of diagnostic criteria, treatment, or outcome in the English literature for over 20 years. We consider patient clinical characteristics, diagnostic criteria, and the usefulness of various diagnostic methods, within the context of a literature review and discussion of a case.

Case Report

Clinical History. The patient, a 36-year-old woman, developed dyspnea, cough, low-grade fever, and night sweats approximately 4 months prior to admission. Initially, she was evaluated at a hospital in another city, without a firm diagnosis. Due to progressive symptoms and the development of nausea and vomiting, along with lower-extremity edema, she presented at our hospital in October of 1992. Previous medical history was noncontributory.

The physical examination at admission demonstrated signs of pericardial tamponade. Chest radiography revealed marked enlargement of the cardiac silhouette. Echocardiography demonstrated a large pericardial effusion, with echocardiographic signs of tamponade. A pericardial window was promptly placed via a subcostal approach. Intraoperative digital exploration of the pericardial space was unremarkable. The removal of effusion fluid resulted in marked relief of tamponade symptoms. Specimens of bloody pericardial fluid and a section of pericardium were negative for malignancy by cytologic and histologic examination, respectively. In addition, cultures and smears (bacteria, acid-fast bacilli, and fungi) were negative. Sections of the pericardium showed non-caseating granulomas. The presumptive diagnosis of an infectious process was made; while awaiting culture results, antibiotic therapy was initiated as recommended by infectious disease consultants.

One week after pericardial window placement, a repeat echocardiogram demonstrated reaccumulation of the pericardial fluid and a mass behind the left ventricle. There were no clinical or echocardiographic signs of recurrent tamponade.

EXHIBIT U

For biopsy of the mass, the patient was advised to undergo a repeat pericardial window procedure via a thoracotomy. She refused.

The 2nd admission, 3 weeks after the 1st, revealed an apparently ill patient with a blood pressure of 112/70, a pulse rate of 108, a respiratory rate of 20, and a temperature of 99 °F. Examination was otherwise notable for estimated right atrial pressures of 12 cm H_2O, no Kussmaul's sign, and no pulsus paradoxus. Upon auscultation, the left hemithorax was dull to percussion, with bibasilar crackles; the point of maximal impulse was diffuse, an S_4 was present, but there was no S_3, no rub, and no knock. Marked hepatomegaly and 3+ pedal edema were present. Repeat echocardiography showed expansion of the pericardial effusion, no echocardiographic signs of tamponade, and an increase in size of the previously noted retrocardiac mass. Computed tomographic scans of the chest (Fig. 1) and abdomen demonstrated bilateral pleural effusions, homogeneous hepatomegaly, and a large pericardial effusion. A mass was noted to be attached to the posterior aspect of the left ventricle, to the left atrium, and to the mediastinal structures superior to the left atrium. There was minimal lymphadenopathy.

Sternotomy was performed, and digital exploration of the pericardial space revealed a tumor encasing the heart. There was initial recovery followed by sudden intractable cardiac arrest, with electromechanical dissociation.

Postmortem Examination. The heart revealed diffuse involvement of the epicardium by a gray-white fleshy tumor having a maximal thickness of 5 cm at the base of the heart. In general, there was a distinct border between the tumor and the underlying myocardium, but areas of obvious myocardial infiltration were observed, most notably at the posterior basal segment of the left ventricle, where myocardium was essentially replaced by tumor (Fig. 2). Tumor packed the pericardial sleeve, investing the roots of the aorta and main pulmonary artery. Within the mediastinum, the tumor was confined by the parietal pericardium. Metastases were noted in the lung, with 4 subpleural nodules ranging up to 1.5 cm in maximum dimension. An organizing thrombus was present within the right atrium, and 2 recent infarcts were noted in the lungs. Acute emboli were present throughout large pulmonary artery branches within the lung parenchyma.

Histopathologic Findings. The general histologic appearance of the tumor consisted of variably sized nodules containing sheets of uniform spindle cells with scanty-to-moderate amounts of eosinophilic cytoplasm and with fusiform-to-oval nuclei having marginated chromatin and inconspicuous nucleoli (Fig. 3). A smaller component of tumor contained cohesive round-to-oval epithelioid cells. Definitive acinar, tubular, or papillary structures were not ob-

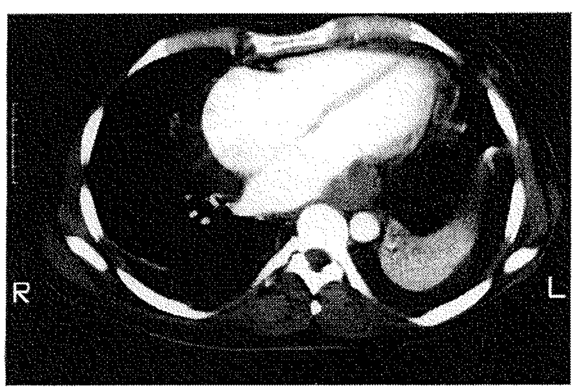

Fig. 1 *Computerized tomographic image of the chest, with contrast. The retrocardiac mass at the level of the atrioventricular groove is well visualized, as is the pericardial effusion. There is also a pleural effusion, and the right atrium appears enlarged.*

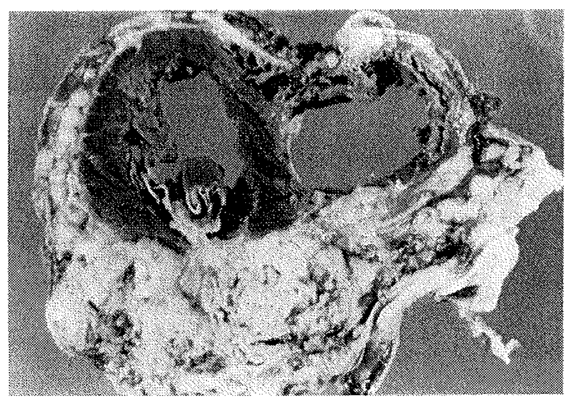

Fig. 2 *Gross pathologic specimen of the heart at the papillary muscle level. The tumor essentially encases the heart. Myocardium is infiltrated and replaced at the base of the heart.*

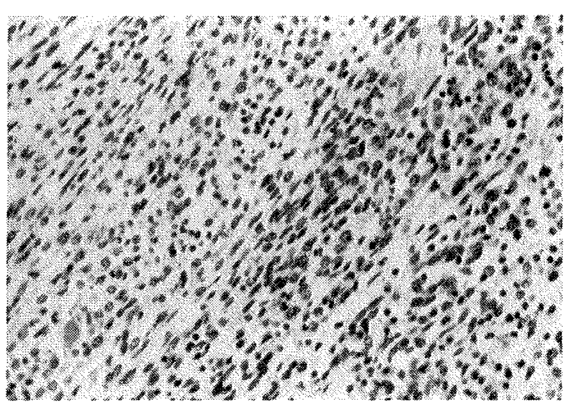

Fig. 3 *Photomicrograph illustrating parallel fascicles of relatively bland oval and spindle cells. (H + E, orig. x400)*

served. The mitotic index varied with location, but areas containing greater than 5 mitoses per 10 high-power fields were present.

Immunohistochemical studies revealed strong vimentin positivity in virtually all tumor cells. Less than 1% of tumor cells demonstrated strong staining for cytokeratin. The remainder of the immunostain battery, including S100, myoglobin, desmin, actin, epithelial membrane antigen, B72.3, and synaptophysin, was negative.

Electron microscopy displayed spindled and oval cells containing generally scanty amounts of rough endoplasmic reticulum, Golgi material, and mitochondria (Fig. 4). The cytoplasmic contours were smooth. Occasional cells contained discontinuous basal lamina material. Primitive cell junctions were observed between clustered oval cells. Although intermediate filaments were quite prominent, neither microfilaments nor microvilli were observed.

Interpretation of Pathologic Findings. The tumor's location and gross pattern of growth, i.e., mostly within the pericardium and forming a rind over the epicardial surface, suggested a diagnosis of mesothelioma. The histologic findings were consistent with the sarcomatoid variant of mesothelioma. However, the relatively undifferentiated spindle cell pattern merited the consideration of sarcomas such as malignant peripheral nerve sheath tumor, leiomyosarcoma, fibrosarcoma, and rhabdomyosarcoma, all of which have been reported as primary cardiac tumors.[1] The biphasic immunostain positivity for vimentin and cytokeratin supported a diagnosis of sarcomatoid mesothelioma. This is the typical pattern displayed by the sarcomatoid and other variants of malignant mesothelioma.[2-5] Most cases of sarcomatoid mesothelioma display positive staining for cytokeratin in >50% of tumor cells, while a smaller number of cases have been reported to display only focal positivity.[5-7] Rare cases are reported to be cytokeratin negative.[3,4] The results of the immunostain battery did not support a nerve sheath, smooth or striated muscle, or fibroblast origin. The ultrastructural features of 10 cases of sarcomatoid mesothelioma were studied by Klima and Bossart,[7] whose findings support a diagnosis of malignant mesothelioma in our tumor, due to the presence of basal lamina material and primitive cell junctions. Microvilli are a prominent ultrastructural finding in the epithelioid and biphasic variants of mesothelioma. However, Klima reported that 5 of their 10 study cases of sarcomatoid mesothelioma revealed no microvilli. Such an absence was observed in our case. The ultrastructural findings were not supportive of alternative diagnoses in the differential diagnosis. Taken together, the pathologic studies provided a definitive diagnosis of primary pericardial sarcomatoid mesothelioma.

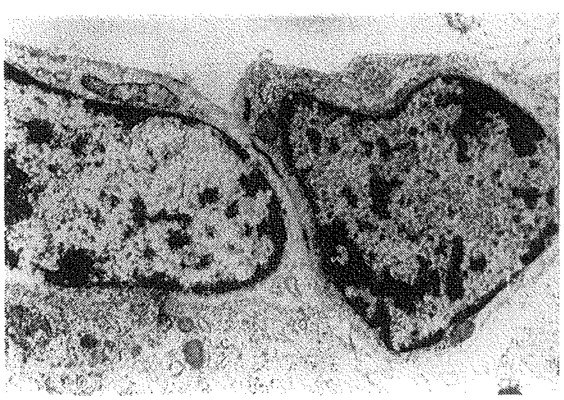

Fig. 4 *Electron micrograph illustrating discontinuous basal lamina and primitive cell junctions. (orig. x26,000)*

Discussion and Literature Review

Pericardial mesothelioma is a rare tumor constituting approximately 4% of the primary heart and pericardial tumors in the Armed Forces Institute of Pathology series[1] and 1% of malignant mesotheliomas in a registry of 180 patients by the Dana Farber Cancer Institute and Brigham and Women's Hospital.[8] The last extensive review of the entity was undertaken in 1971 by Sytman and MacAlpin.[9] In order to update the available knowledge concerning its clinical and pathological features, we reviewed 24 literature citations of 27 cases in which a diagnosis of primary pericardial mesothelioma was made.[10-33] These citations appeared in the English literature from 1972 through 1992. The findings of this review, combined with the findings in our case, are summarized in Tables I-IV.

Table I illustrates that for the 28 cases, there is a male–female ratio of 2:1. Although a wide age range is affected, over half of the cases occur in the 5th to the 7th decades. Presenting signs and symptoms are nonspecific, and are related mostly to the compromise of cardiac function caused by tumor mass, effusion, or both. This nonspecificity may lead to diagnostic consideration or treatment of other disease states associated with pericardial effusion, such as rheumatic fever,[13] metastatic disease,[26] dissecting aortic aneurysm,[15] viral syndrome,[20] collagen vascular disease,[11,21,25] and tuberculosis.[31] Due to extension of tumor into the myocardium and cardiac chambers, acute myocardial infarction or cerebral vascular accident may occur during the course of the disease.[17,21,33] The role of asbestos exposure as a causative factor in the development of pericardial mesothelioma is not certain, but asbestos exposure has been documented in a few patients.[16,18,34]

TABLE I. Reported Characteristics and Presenting Symptoms of Patients*

Sex		
Male	20/28	(71%)
Female	8/28	(29%)
Age		
Range	12-77 years	
Median	48 years	
Mean	47 years	
Most Commonly Cited Presenting Symptoms		
Dyspnea	13/28	(46%)
Fever	9/28	(32%)
Chest pain	9/28	(32%)
Weight loss	6/28	(21%)
Asbestos Exposure		
Not mentioned	16/28	(57%)
Exposure	4/12	(33%)
No known exposure	8/12	(67%)

*Drawn from references 10 through 33 and the present case.

Although chest radiography and echocardiography are sensitive means of detecting enlarged cardiac silhouette and effusion, Table II illustrates that they lack sensitivity in detection of pericardial mesothelioma. This likely is due to the tendency of this tumor to exhibit a diffuse growth pattern (Table III) in which tumor envelopes the heart, rather than a pattern of growth as a discrete mass. Computed tomography appears to offer a greater sensitivity for detection of the tumor.[15,20,22,25,35] In the 2 reports in which it was mentioned, magnetic resonance imaging successfully identified tumor.[22,27] Finally, radionuclide imaging (with radiogallium) was reported to have been of assistance in the detection of tumor in 2 cases.[22,25]

Table III summarizes the pathologic findings of the cases. A diffuse growth pattern is most common, and myocardial invasion is not uncommon. Involvement of the mitral valve resulting in valve dysfunction was noted in 2 cases and led to initial misinterpretations of the cause—as rheumatic heart disease[13] and atrial myxoma.[24] The results of cytologic study of effusions, given in 10 cases, suggest this to be an insensitive means of detecting tumor, since only 30% of these cases demonstrated malignant or atypical cells. The 20 cases in which the microscopic pattern of the tumor is reported reveal an even distribution over the 3 histologic variants of mesothelioma.

Pericardial mesothelioma is a highly lethal disease (Table IV). In 1 of the 28 cases, a possible cure was achieved by surgical excision of a discrete tumor mass attached to the parietal pericardium.[30] Com-

TABLE II. Summary of Reported Radiographic Findings in Pericardial Mesothelioma*

Chest Radiography		
Results not available	4/28	(14%)
Results available	24/28	(86%)
Normal	4/24	(17%)
Enlarged cardiac silhouette	16/24	(67%)
Mediastinal widening	1/24	(4%)
Anterior mediastinal mass	1/24	(4%)
Echocardiography		
Not done or results not available	12/28	(43%)
Results available	16/28	(57%)
Effusion	14/16	(88%)
Tamponade	3/16	(19%)
Thickened pericardium	3/16	(19%)
Mitral valve abnormality	2/16	(12%)
Pericardial mass	2/16	(12%)
Pericardial cysts instead of mass	1/16	(6%)
Pericardial adhesions instead of mass	1/16	(6%)
Computed Tomography		
Not done or results not available	19/28	(68%)
Results available	9/28	(32%)
Pericardial mass	4/9	(44%)

*Drawn from references 10 through 33 and the present case.

TABLE III. Summary of Pathologic Findings*

Gross Findings		
Diffuse growth	18/25	(72%)
Discrete mass(es)	7/25	(28%)
Not available	3/28	(11%)
Myocardial invasion	18/28	(64%)
Extra-mediastinal metastases	7/28	(25%)
Effusion Cytologic Findings		
Not available	18/28	(64%)
Benign	7/10	(70%)
Inconclusive	1/10	(10%)
Malignant	2/10	(20%)
Histopathologic Findings		
Not available	8/28	(28%)
Biphasic	7/20	(35%)
Epithelioid	7/20	(35%)
Sarcomatous	6/20	(30%)

*Drawn from references 10 through 33 and the present case.

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TABLE IV. Status of Patients at Time of Article's Publication*

Not available	1/28	(4%)
Dead of disease	24/27	(89%)
Alive with disease	2/27	(7%)
Alive without evidence of disease	1/27	(4%)

*Drawn from references 10 through 33 and the present case.

plete excision was not possible in the remainder of the patients, although partial excision was undertaken in a number of cases. This appeared to be of temporary benefit in selected patients.[14,22,27,31] Chemotherapy and radiation therapy appear to be of little benefit in cases of non-resectable tumor.[24,28-30,32] In 20 fatal cases, the time course of disease was documented and the patients lived an average of 3.5 months after presentation or initiation of therapy.

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Exhibit V

Int J Cardiovasc Imaging (2010) 26:625–627
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CASE IN POINT

Surprising finding of a primary pericardial mesothelioma

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Abstract In this report, we describe a case of primary pericardial mesothelioma, which is an extremely rare tumor arising from mesothelial cells lining the pericardium and is associated with a dismal prognosis.

Keywords Primary pericardial mesothelioma · Pericardial disease · Cardiac masses

A 76-year-old man without a history of cardiac disease was admitted with gradual decline in mental status. Magnetic resonance imaging (MRI) of the brain revealed small lesions suspicious of embolic events, which prompted cardiac evaluation. His chest radiograph showed an enlarged cardiac silhouette with bibasilar atelectasis and a small left pleural effusion. A 12-lead electrocardiogram demonstrated new atrial fibrillation with diffuse ST-segment elevation suggestive of pericarditis. Transthoracic echocardiography showed a complex mass, possibly in the pericardial space surrounding the right ventricle and apex without a significant pericardial effusion.

Cardiac MRI (Figs. 1, 2) demonstrated a large multinodular mass within the pericardial space most consistent with a tumor encasing the heart, systemic, and pulmonary veins without critical compression. The mass appeared to be heterogeneous in nature with the central portions of the nodules having higher water content and being avascular, consistent with a possibly necrotic, partially liquefied core. Importantly, a clear demarcation of tumor edge from normal myocardium was noted, suggesting absence of deep tumor invasion into the myocardium.

The differential diagnosis for tumors affecting the pericardium includes metastases to the pericardium from carcinomas of other chest organs, hematologic malignancies, melanoma, and rarely primary cardiac tumors [1]. The fact that the tumor was located predominantly in the pericardial space without pathological findings in the lungs, mediastinum, pleura, or myocardium renders metastatic disease from the lungs, melanoma, or hematologic malignancies less likely. In particular, the patient did not have a primary pleural mass. Sarcomas, which as a group are the most frequent malignant cardiac tumors, are

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EXHIBIT Z

EXHIBIT V

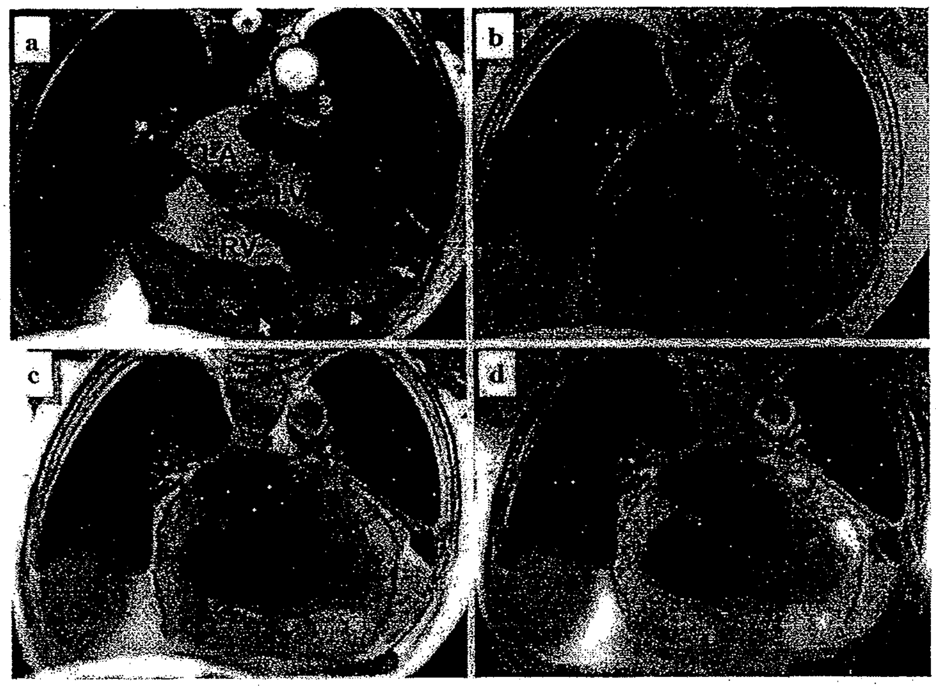

Fig. 1 *Cine MR imaging* (steady-state free precession) for dynamic assessment demonstrated partial compression of the ventricular cavity by the bulk of the mass (*arrows*) (**a**). There was no valvular pathology or intracavitary mass noted, to explain the embolic phenomena (diastolic frames are shown; *LA* left atrium, *LV* left ventricle, *RV* right ventricle). *Tomographic MR images* revealed a nodular tumor in the pericardial space encasing the heart and pulmonary veins. On T1-weighted fast spin echo imaging (**b**), the mass was homogenous and relatively isointense with myocardium. On T2-weighted fast spin echo imaging (**c**), the mass was heterogeneous with the nodules being hyperintense relative to myocardium, suggesting higher water content of the nodular structures. T2-weighted imaging with fat suppression (**d**), excluded a tumor with substantial fat content. A small left-sided pleural effusion without pleural thickening was also noted

typically bulky, infiltrating masses originating from the myocardial wall and may involve the pericardium secondarily.

A CT-guided needle core biopsy (Fig. 2d) of the pericardial mass showed a spindle cell neoplasm with moderate cellularity and nuclear atypia. Diffuse immunohistochemical staining for cytokeratin confirmed the epithelial origin of the tumor and, taken together with its morphology and distribution, allowed for a diagnosis of primary sarcomatoid malignant mesothelioma. The patient had a history of remote occupational asbestos exposure. After the diagnosis was made, the patient opted for palliative care measures only and died at home a week later.

Prognosis of primary pericardial mesothelioma is poor with median survival of less than 6 months, though the use of radical surgery, radiotherapy, adjuvant chemotherapy and immunomodulation have led to survival up to 16 months [2, 3]. Our report describes the imaging characteristics of this extremely rare tumor. It is unclear, whether the tumor was associated with the patients presenting symptoms (possibly by increased coaguability) or was a truly unrelated finding.

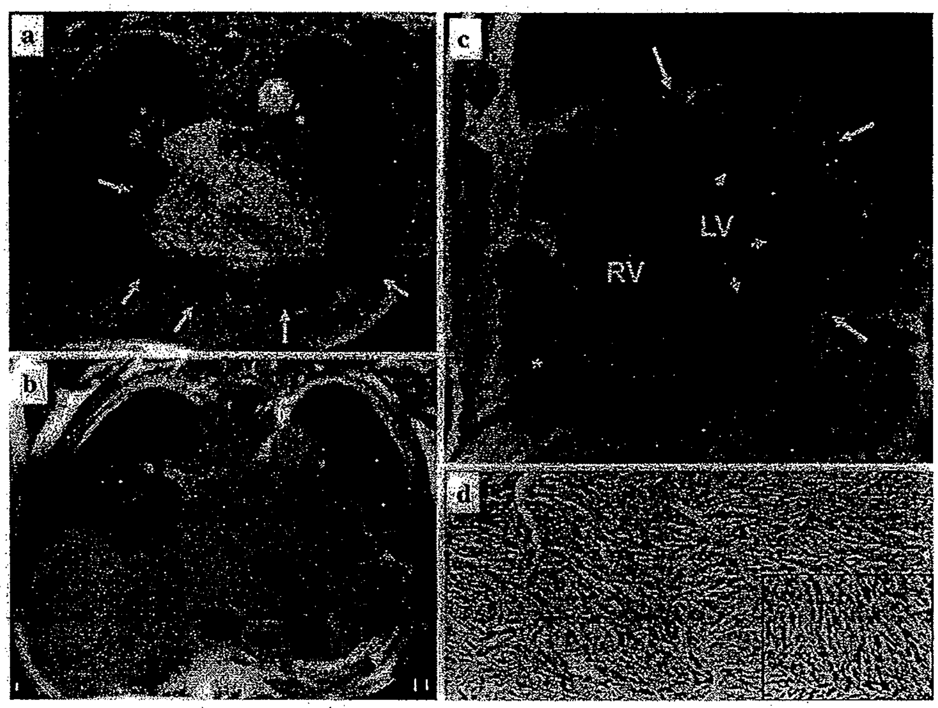

Fig. 2 *First-pass MR perfusion* with gadolinium contrast (**a**) demonstrated heterogeneous vascularity of the mass with little or no perfusion (*dark regions, arrows*) of the central zones of the tumor nodules. *Early post-contrast imaging* (inversion-recovery gradient-echo) with a long inversion time of 600 ms (**b**) showed continued lack of contrast uptake 5 min later. *Delayed contrast-enhancement images* (**c**) obtained 10 min after injection of gadolinium contrast with inversion-time set to null signal from normal myocardium demonstrated a heterogeneous appearance of the tumor (*RV* right ventricle, *LV* left ventricle). Some areas of the tumor were predominantly bright (*arrows*) indicating contrast material uptake, while the major nodules were *grey* with a *black* "etched" border (*asterisk*) reflecting lack of contrast uptake. There was a clear demarcation of the tumor from normal myocardium (*arrowheads*), thus grossly appreciable, deep tumor invasion into the myocardium was not seen in this patient. On histologic examination (**d**) tumor cells are arranged in a poorly defined pattern with interspersed capillaries and mitoses (hematoxylin-eosin, original ×100). Diffuse cytoplasmic staining for cytokeratin in sarcomatoid mesothelioma (*small insert*: pan-cytokeratin immunohistochemistry, original ×200)

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Exhibit W

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RESEARCH ARTICLE

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Malignant mesothelioma following repeated exposures to cosmetic talc: A case series of 75 patients

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Abstract

Background: Asbestos is the primary known cause of malignant mesothelioma. Some cosmetic talc products have been shown to contain asbestos. Recently, repeated exposures to cosmetic talc have been implicated as a cause of mesothelioma.

Methods: Seventy-five individuals (64 females; 11 males) with malignant mesothelioma, whose only known exposure to asbestos was repeated exposures to cosmetic talcum powders, were reviewed in medical-legal consultation. Out of the 75 cases, 11 were examined for asbestiform fibers.

Results: All subjects had pathologically confirmed malignant mesothelioma. The mean age at diagnosis was 61 ± 17 years. The mean latency from exposure to diagnosis was 50 ± 13 years. The mean exposure duration was 33 ± 16 years. Four mesotheliomas (5%) occurred in individuals working as barbers/cosmetologists, or in a family member who swept the barber shop. Twelve (16%) occurred in individuals less than 45 years old (10 females; 2 males). Forty-eight mesotheliomas were pleural (40 females; 8 males), 23 were peritoneal (21 females; 2 males). Two presented with concomitant pleural and peritoneal disease. There was one pericardial, and one testicular mesothelioma. The majority (51) were of the epithelioid histological subtype, followed by 13 biphasic, 8 sarcomatoid, 2 lymphohistiocytoid, and 1 poorly differentiated. Of the 11 individuals whose nontumorous tissues were analyzed for the presence of asbestiform fibers, all showed the presence of anthophyllite and/or tremolite asbestos.

Conclusions: Mesotheliomas can develop following exposures to cosmetic talcum powders. These appear to be attributable to the presence of anthophyllite and tremolite contaminants in cosmetic talcum powder.

KEYWORDS

anthophyllite, females, mesothelioma, peritoneal, pleural, talc, tremolite

1 | INTRODUCTION

Asbestos, a generic term for naturally occurring fibrous mineral silicates, is recognized as a carcinogen by the general medical and scientific communities. In 1960, Wagner et al[1] reported a large series of malignant mesotheliomas in individuals who had been exposed to asbestos from a South African asbestos mine. It has been demonstrated that all types of asbestos and even brief and low-dose exposures are capable of causing malignant mesothelioma.[2-4] In the 1970s, several types of cosmetic talcum powder products were

EXHIBIT W

demonstrated to contain asbestos.[5-7] Asbestos fibers in commercial talcum powder have also been shown to become airborne upon application, and repeated exposures to cosmetic talc were implicated as a cause of mesothelioma by Gordon et al.[8] Recently, Moline et al,[9] reported a series of 33 subjects with malignant mesothelioma, whose only known exposure to asbestos was cosmetic talc. We present 75 additional subjects, with malignant mesothelioma, whose only known exposure to asbestos was cosmetic talc.

2 | METHODS

One hundred forty subjects with documented exposures to cosmetic talc were initially reviewed. Exposures were identified through sworn deposition testimonies and answers to sworn interrogatories provided from subjects, parents, and spouses. Sixty-five subjects were excluded due to recalled occupational or paraoccupational exposures to other sources of asbestos. Seventy-five subjects, whose only known exposure to asbestos was via cosmetic talc, were included for further examination. The asbestos content of talcum products and airborne asbestos concentrations during simulations of the usage of these products was determined in previously published studies.[10,11]

Tissues from biopsies and/or debulking procedures were examined and the diagnosis of malignant mesothelioma was confirmed by a board-certified pathologist (JCM, TSE, RLK). Immunohistochemical staining results for BAP-1 were available in a few cases but was not routinely performed as a part of this study.

No efforts were made to reconstruct levels of exposure but all subjects had been repeatedly exposed over many years. Eleven cases were examined for the presence of asbestiform fibers (aspect ratio, ≥3:1) in sampled tissues. Nine subjects were examined both by analytical transmission electron microscopy (ATEM) and microprobe analysis (MA) (see Table 2), whereas two were examined by scanning electron microscopy (SEM) and MA (results not shown).

3 | RESULTS

The pertinent data from the 75 subjects is shown in Table 1. All had pathologically confirmed malignant mesothelioma. Sixty-four subjects were females, 11 were males. The mean age at diagnosis was 61 ± 17 years, with a range of 14 to 94 years. The mean exposure duration was 33 ± 16 years with a range of exposure from 6 to 65 years. The mean latency from time of first exposure to diagnosis was 50 ± 13 years with a range of 14 to 72 years. A total of 4 of the 75 cases (5%) occurred in barbers/cosmetologists, or in a family member who swept the barber shop. Twelve (16%) were 45 years old or younger (10 females, 2 males) at the time of diagnosis. Forty-eight mesotheliomas were pleural (40 females; 8 in males); 23 peritoneal (21 females; 2 men). Two presented with both pleural and peritoneal disease. There was one pericardial (woman), and one testicular mesothelioma. The majority, 51 (68%) were of epithelioid subtype, 13 biphasic (17%), 8 sarcomatoid (11%), 2 lymphohistiocytoid (3%), and 1 poorly differentiated (1%). Treatment, therapeutic outcomes, and survival were not determined in this study.

For the 11 subjects whose tissues were examined by ATEM and ASEM, the analysis showed the presence of tremolite and/or anthophyllite in all 11 subjects (Table 2).

4 | DISCUSSION

The 75 individuals with malignant mesothelioma caused by asbestos in cosmetic talc is currently the largest series reported to date. Recently, Moline et al reported 33 cases of malignant mesothelioma attributed to exposures to cosmetic talc. Like Moline's work, most of mesotheliomas in the present series occurred in women. Several mesotheliomas occurred specifically in hairdressers/barbers. Similarly, the asbestos fiber types found by ATEM in the tissues examined were comparable to those found in laboratory testing for cosmetic talc.[10-12]

Mesothelioma is recognized as a "signal tumor" of asbestos exposure, that is, if a patient has mesothelioma, it should signal an inquiry into potential asbestos exposure. The presence of asbestos in talc deposits has been recognized since the late 1940s.[13,14] Since the 1960s, laboratory testing has identified asbestos in samples of cosmetic talc.[15,16] Studies have confirmed that the most common types of asbestos present in cosmetic talc are tremolite, anthophyllite, and chrysotile. Industrial asbestos products used in the United States generally contained chrysotile, amosite, and/or crocidolite,[17] and anthophyllite and tremolite were rarely present.[18]

While the latency between exposure and diagnosis in the present study is similar to the average latency for the development of mesothelioma (50 years) reported in surveillance epidemiology and end results program (SEER) data,[19] the average age at diagnosis in this report (61 years) is 11 years younger than that in the SEER data (72 years). In addition, fewer than 3% of mesotheliomas in the SEER data occurred in individuals less than 45 years of age, whereas 16% of mesotheliomas of the present study occurred in individuals less than 45 years of age, and 83% of these cases were in women.[20]

The present report of 75 cases, together with the 35 cases previously reported[8,9] currently brings the number of individuals with confirmed diagnoses of malignant mesothelioma following repeated exposure to cosmetic talcum powder to more than 100. The presence of anthophyllite and tremolite in the fiber analysis of tissues obtained from the 11 subjects in this series, is consistent with a source in cosmetic talc.

Unlike industrial or occupational exposure to asbestos, where materials have been regulated, exposure to asbestos in cosmetic talc has not been widely reported or recognized within the medical community or to the public. Cosmetic talc products are most frequently used by women in the United States, and while the incidence of mesothelioma in women is less than in men, the majority have previously been reported as "idiopathic," indicating no recognized source of asbestos exposure. The present study supports the contention that asbestos exposure through the use of cosmetic talc accounts may account for an uncertain percentage of these cases.

TABLE 1 Seventy-five mesothelioma cases exposed to talcum powder

Case	Sex	Year of diagnosis	Age at diagnosis	Mesothelioma site	Histology	Estimated years of use	Estimated years of latency
1	F	2017	72	Pleural	Epithelioid	20	57
2	F	2014	51	Peritoneal	Epithelioid	30	50
3	F	2017	50	Pleural	Lymphohistiocytoid	41	50
4	F	2017	57	Peritoneal	Epithelioid	30	52
5	F	2015	65	Pleural	Epithelioid	39	62
6	F	2017	39	Peritoneal	Sarcomatoid	15	39
7	F	2016	29	Pericardial	Epithelioid	29	29
8	F	2017	94	Pleural	Epithelioid	60	72
9	F	2015	80	Pleural	Epithelioid	19	59
10	F	2016	72	Pleural	Sarcomatoid	43	59
11	F	2013	66	Peritoneal	Epithelioid	20	52
12	F	2011	48	Pleural	Lymphohistiocytoid	13	21
13	F	2010	51	Peritoneal	Epithelioid	15	20
14	F	2018	55	Peritoneal	Epithelioid	40	42
15	M	2017	81	Pleural	Sarcomatoid	60	60
16	F	2018	56	Pleural	Epithelioid	48	52
17	F	2017	32	Peritoneal	Epithelioid	25	32
18	F	2017	89	Pleural	Sarcomatoid	40	42
19	F	2019	73	Peritoneal	Epithelioid	47	56
20	M	2016	70	Pleural	Poorly differentiated	50	55
21	F	2015	66	Pleural	Epithelioid	40	43
22	F	2016	45	Pleural	Epithelioid	10	45
23	F	2018	45	Peritoneal	Epithelioid	39	45
24	M	2015	67	Pleural + peritoneal	Epithelioid	35	60
25	M	2017	78	Peritoneal	Biphasic	50	62
26	F	2018	57	Peritoneal	Biphasic	25	57
27	F	2013	14	Peritoneal	Epithelioid	12	14
28	F	2016	67	Peritoneal	Epithelioid	15	59
29	F	2018	73	Pleural	Epithelioid	30	65
30	F	2018	76	Pleural	Biphasic	60	55
31	M	2017	39	Testis	Epithelioid	7	39
32	F	2018	57	Pleural	Sarcomatoid	57	57
33	F	2016	68	Pleural	Epithelioid	38	64
34	F	2017	80	Pleural	Epithelioid	50	60
35	F	2016	63	Pleural	Epithelioid	15	54
36	F	2017	58	Pleural	Biphasic	20	58
37	F	2017	71	Pleural	Biphasic	60	71
38	F	2014	70	Pleural	Epithelioid	41	39
39	F	2016	26	Peritoneal	Epithelioid	20	26

TABLE 1 (Continued)

Case	Sex	Year of diagnosis	Age at diagnosis	Mesothelioma site	Histology	Estimated years of use	Estimated years of latency
40	F	2016	35	Pleural	Epithelioid	35	35
41	F	2017	72	Pleural	Sarcomatoid	23	60
42	F	2016	68	Peritoneal	Epithelioid	65	68
43	F	2018	77	Pleural	Biphasic	30	55
44	M	2015	58	Plural	Biphasic	6	49
45	F	2017	72	Peritoneal	Biphasic	30	42
46	F	2017	59	Pleural + peritoneal	Epithelioid	15	44
47	F	2016	80	Pleural	Biphasic	16	52
48	M	2019	71	Pleural	Epithelioid	40	57
49	F	2017	72	Pleural	Biphasic	58	58
50	F	2017	43	Peritoneal	Epithelioid	43	43
51	F	2017	75	Peritoneal	Sarcomatoid	55	59
52	F	2015	30	Pleural	Epithelioid	20	20
53	F	2017	79	Pleural	Biphasic	65	61
54	F	2017	66	Peritoneal	Epithelioid	20	60
55	F	2015	64	Peritoneal	Epithelioid	40	40
56	F	2017	24	Pleural	Epithelioid	12	24
57	M	2017	72	Pleural	Epithelioid	30	56
58	M	2017	74	Peritoneal	Epithelioid	30	52
59	M	2015	30	Pleural	Epithelioid	20	30
60	F	2016	81	Pleural	Sarcomatoid	52	52
61	F	2017	58	Pleural	Epithelioid	58	58
62	F	2016	75	Pleural	Epithelioid	8	47
63	F	2011	88	Pleural	Epithelioid	21	71
64	F	2016	73	Peritoneal	Biphasic	41	60
65[a]	M	2017	64	Pleural	Epithelioid	18	40
66[a]	F	2014	69	Pleural	Epithelioid	16	60
67[a]	F	2014	44	Peritoneal	Epithelioid	30	39
68[a]	F	2016	68	Pleural	Epithelioid	53	52
69[a]	F	2016	72	Pleural	Epithelioid	40	51
70[a]	F	2016	67	Pleural	Epithelioid	37	53
71[a]	F	2017	58	Pleural	Epithelioid	41	46
72[a]	M	2016	44	Pleural	Epithelioid	43	44
73[a]	F	2017	51	Pleural	Epithelioid	28	49
74[a]	F	2015	47	Pleural	Epithelioid	15	40
75[a]	F	2014	62	Pleural	Biphasic	14	53

[a]Tissue analysis performed.

The present study has several limitations. It is both retrospective and uncontrolled, and the cases were submitted in medico-legal consultation, all of which potentially introduce bias. However, detailed deposition testimonies provide a level of detail concerning product exposure—including dates of exposure, duration, and frequency—that is rarely obtained in routine medical exposure histories, and which allowed for corroborating witness testimony in some cases. The strengths of the current series include its size, as malignant mesothelioma is a rare disease

TABLE 2 Fiber detection in tissue digestion from nine cases of malignant mesothelioma

Case	Mesothelioma site	Asbestos type	Tissues examined	Concentration (fibers per gram of wet tissue) Lung, lymph node, omentum, ovary	Limit of detection (fibers per gram of wet tissue) Lung, lymph node, omentum, ovary	Tissue digest weight (g) Lung, lymph node, omentum, ovary
65	Pleural	Anthophyllite, tremolite	Lung, lymph node	8625	4313	0.08, 0.34
66	Pleural	Anthophyllite	Lung, lymph node	15 333, 23 000	7667, 1150	0.06, 0.06
67	Peritoneal	Anthophyllite, tremolite	Omentum, lymph node	1917, 1725	639, 1725	0.54, 0.20
68	Pleural	Anthophyllite, tremolite	Lymph node	3044	1015	0.82, 0.34
70	Pleural	Anthophyllite, amosite, chrysotile	Lymph node	17 250	3450	1.06
71	Pleural	Anthophyllite, tremolite	Lung, lymph node	4313, 857, 3451	2156, 857, 575	0.16
72	Pleural	Anthophyllite, tremolite	Lymph node	17 250	3450	0.02
74	Pleural	Anthophyllite, tremolite	Lung	2300	460	2
75	Pleural	Anthophyllite	Lung, ovary	3450, 2070	1150, 2070	0.6, 0.2

Note: All cases shown were examined by analytical transmission electron microscopy and structures analyzed by microprobe analysis.

(1-2 cases per 100 000), and its novelty, as exposures to cosmetic talc are rarely considered by most medical practitioners when they are eliciting an exposure history to asbestos.

The findings of the present and other recent studies suggest that cosmetic talc may be a cause of malignant mesothelioma. Large-scale controlled studies will be required to assess the prospective risk of developing mesothelioma following repeated exposures to talc. Although cosmetic talcs are not currently regulated by the Food and Drug Administration, the poor prognosis of malignant mesothelioma may warrant regulation or the withdrawal of cosmetic talcs from the market, as nontoxic alternatives such as corn starch are presently available.

CONFLICTS OF INTEREST

Drs Emory, Maddox, and Kradin have testified in asbestos litigation, primarily for plaintiffs.

DISCLOSURE BY AJIM EDITOR OF RECORD

John D. Meyer declares that he has no conflict of interest in the review and publication decision regarding this article.

AUTHOR CONTRIBUTIONS

JCM and RLK developed the concept and the design of the work. JCM initiated the acquisition and developed the initial data analysis. TSE reviewed the materials, performed the statistical analysis, and was the primary author of the manuscript. RLK revised and gave the final approval of the version to be published.

ETHICS APPROVAL AND INFORMED CONSENT

As these cases were selected from medical-legal consultation practice and no identifying information was included, there was no formal institutional consent nor informed consent required.

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Exhibit X

Original article

Scand J Work Environ Health 2020;46(6):609-617

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Association between asbestos exposure and pericardial and tunica vaginalis testis malignant mesothelioma: a case-control study and epidemiological remarks

by Marinaccio A, Consonni D, Mensi C, Mirabelli D, Migliore E, Magnani C, Di Marzio D, Gennaro V, Mazzoleni G, Girardi P, Negro C, Romanelli A, Chellini E, Grappasonni I, Madeo G, Romeo E, Ascoli V, Carrozza F, Angelillo IF, Cavone D, Tumino R, Melis M, Curti S, Brandi G, Mattioli S, Iavicoli S; ReNaM Working Group

Using Italian national mesothelioma registry data and a case-control study design, this study provides evidence for the first time of the association between asbestos exposure and pericardial and tunica vaginalis testis mesothelioma. Our findings confirm the extreme rarity and poor survival of these diseases and support the causal role of asbestos for all mesotheliomas.

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Key terms: asbestos; case–control study; epidemiology; exposure; Italy; malignant mesothelioma; mesothelioma; national registry; pericardial and tunica vaginalis testis; rare disease

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Additional material

Please note that there is additional material available belonging to this article on the *Scandinavian Journal of Work, Environment & Health* -website.

EXHIBIT X

Original article

Scand J Work Environ Health. 2020;46(6):609–617. doi:10.5271/sjweh.3895

Association between asbestos exposure and pericardial and tunica vaginalis testis malignant mesothelioma: a case–control study and epidemiological remarks

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Objectives The purposes of this study are to describe the epidemiology of pericardial and tunica vaginalis testis mesothelioma and assess the role of asbestos exposure for these rare diseases.

Methods Based on incident pericardial and tunica vaginalis testis mesothelioma cases collected from the Italian national mesothelioma registry (ReNaM) in the period 1993–2015, incidence rates, survival median period and prognostic factors have been evaluated. A case–control study has been performed to analyze the association with asbestos exposure (occupational and non-occupational) for these diseases.

Results Between 1993 and 2015, 58 pericardial (20 women and 38 men) and 80 tunica vaginalis testis mesothelioma cases have been registered with a mean annual standardized (world standard population as reference) incidence rates of 0.049 (per million) in men and 0.023 in women for the pericardial site, and 0.095 for tunica vaginalis testis mesothelioma. Occupational exposure to asbestos was significantly associated with the risk of the diseases [odds ratio (OR) 3.68, 95% confidence interval (CI) 1.85–7.31 and OR 3.42, 95% CI 1.93–6.04 in pericardial and tunica vaginalis testis mesothelioma, respectively]. The median survival was 2.5 months for pericardial and 33.0 months for tunica vaginalis testis mesotheliomas. Age was the main predictive factor for survival for both anatomical sites.

Conclusions For the first time in an analytical study, asbestos exposure was associated with pericardial and tunica vaginalis testis mesothelioma risk, supporting the causal role of asbestos for all anatomical sites. The extreme rarity of the diseases, the poor survival and the prognostic role of age have been confirmed based on population and nationwide mesothelioma registry data.

Key terms epidemiology; Italy; national registry; rare disease.

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Malignant mesothelioma (MM) is a neoplasm of mesothelial cells, and the International Agency for Research on Cancer (IARC) has firmly established the causal role of asbestos (1–3). Despite international health institutes' and agencies' recommendations (4–7), currently 2 000 000 tons of asbestos per year are still produced worldwide (8, 9). Pericardial and tunica vaginalis of testis (TVT) MM accounted for around 1% of cases in the available MM case series (10–13). Incidence and survival for these forms have seldom been reported. The surveillance, epidemiology, and end results (SEER) program from the US National Cancer Institute provided evidence of mean annual standardized incidence rates, in 1973–2013, for pericardial MM of 0.35 and 0.36 (per 10 million person-years) in men and women, respectively, and of 0.54 for TVT MM (14).

The causal role of asbestos exposure in pericardial and TVT MM aetiology has been considered as plausible, but no epidemiological analytical study ever tested the asbestos (or other putative risk factors) role for these diseases (11, 12, 15, 16). Italy is one of the countries more widely involved in the current epidemic of asbestos-related diseases due to the large use of asbestos in the past and the number of exposed individuals among workers (and in the general population) until the asbestos ban issued in 1992 (17–20). In this context, a permanent and mandatory epidemiologic surveillance system on MM is active, based on a national MM registry (Registro Nazionale dei Mesoteliomi, ReNaM in Italian). ReNaM aims are to provide estimates of MM incidence at national population level to assess and record asbestos exposures of cases and identify any possible underestimated or unknown source of asbestos contamination.

This study aims to (i) describe incidence and survival for pericardial and TVT MM cases detected by ReNaM and (ii) assess the association with asbestos exposure using a case-control design.

Methods

Incidence and survival analyses

ReNaM is an epidemiological surveillance system characterized by a network of regional operating centers [Centri Operativi Regionali (COR) in Italian] gradually established by all 20 Italian regions. Case series from Calabria, Sardinia and Molise are available but these regions still cannot ensure completeness of registration. Reporting is compulsory, but COR also actively search and register incident MM cases at the healthcare services that diagnose and treat most cases (pneumology and chest surgery units as well as pathology units). Completeness of registration is periodically checked by surveys, using regional current health sources, pathology units, hospital admissions and mortality registries. According to ReNaM guidelines (21) and as extensively described elsewhere (22), cases are classified as "definite" (histological confirmation of diagnosis, possibly completed by immunohistochemical characterization, and confirmation by imaging and clinical diagnosis), "probable" (usually, cytological diagnosis and confirmation by imaging and clinical diagnosis) or "possible" MM (clinical diagnosis with positive imaging). The occupational and residential history and information on lifestyle habits are obtained using a standardized questionnaire administered by trained interviewers to cases or their next of kin. COR assess both occupational and non-occupational exposure to asbestos. Occupational histories are coded using the Italian standard classifications of industry and occupation. In each COR, experts carry out the exposure assessment in cooperation, if necessary, with the industrial health and safety units of local health authorities. Lifetime asbestos exposure is classified as "occupational" (definite, probable, possible) or "non-occupational" (familial, environmental, other non-occupational – such as leisure-time-related

activities), according to the ReNaM guidelines (21). "Unlikely" exposure is assigned to subjects for whom information is inadequate or asbestos exposure could be reasonably ruled out.

Actually, ReNaM has collected cases with a diagnosis of MM from 1993–2015. Pericardial and TVT MM cases were extracted and analyzed for the whole period. Age and gender standardized incident rates have been calculated using the world, European (as proposed by Eurostat in 2013) and Italian (2011 census) populations as standard populations. The distribution and the extent of person-years of observations is reported in the supplementary material (www.sjweh.fi/show_abstract.php?abstract_id=3895) table S1. Survival was estimated by the Kaplan-Meier method, and Cox's proportional hazard regression has been used to assess the role of prognostic factors separately for pericardial and TVT MM. The predictive variables in the final Cox's multivariate model were gender (men and women), age at diagnosis (categorized as follows: ≤64, 65–74, ≥75 years old), calendar period of diagnosis (1993–2003 and 2004–2015), diagnosis level of certainty (definite, probable and possible MM) and morphology (epithelioid, biphasic, fibrous, unspecified MM). The reference modality was the first for all predictive variables in the model. The model's goodness-of-fit to empirical data was assessed by the log likelihood test.

Case–control study

We conducted a case–control study using (i) pericardial and TVT MM cases registered by ReNaM in the period 1993–2015, during which coverage of the Italian population progressed as previously described, and (ii) two sets of controls recruited in two earlier case–control studies: a population-based study on pleural mesothelioma and a hospital-based study on cholangiocarcinoma.

We only used controls who had completed the questionnaire. The distribution of controls by gender, age, year of birth, and region of residence is described in table S3. The first set of controls was taken from a multicentric unpublished population-based case–control study on pleural mesothelioma (called MISEM), performed in five Italian regions (Apulia, Lombardy, Piedmont, Tuscany, and Veneto). Controls were frequency-matched to cases by gender and age and randomly sampled from residents aged 20–89 years in 2012 (Apulia, Piedmont, Tuscany and Veneto) and 2014 (Lombardy). Interviews were performed in 2014–2015 and the participation rate was 48.4%. The second set of controls was taken from a hospital-based unpublished case–control study on cholangiocarcinoma performed in Emilia-Romagna region (called CARA) in which controls were enrolled and interviewed in 2014–2016, and that was an evolution of a previous epidemiological study (23). Participation rate was almost complete. Both sets of controls were interviewed using the same standardized ReNaM questionnaire and assessment of asbestos exposure was performed following ReNaM guidelines.

We calculated odds ratios (OR) and 95% confidence intervals (CI) of pericardial and TVT MM for lifetime asbestos exposure (occupational, familial, environmental, and leisure activity related) using "unlikely" asbestos exposure as reference. To avoid sparse data problems, we fitted conditional regression models using age categories (<55, 55–59, 60–64, 65–69, 70–74, 75–79, and ≥80 years) as the adjustment set (24). For pericardial MM, we calculated gender-specific and -adjusted analyses. Sensitivity analyses were performed by applying the following restrictions to cases: (i) only cases from the six regions which enrolled control subjects (table S4); (ii) only cases with definite diagnosis (table S5); (iii) only cases diagnosed in 2000–2015 (table S6); 4) only subjects born before year 1950 (table S7). Finally, we performed specific analyses by economic sectors of exposure. Statistical analyses were performed with Stata version 15, 2017 (StataCorp, College Station, TX, USA).

Results

Incidence and survival analyses

Between 1993 and 2015, 58 pericardial MM cases (38 and 20 in men and women, respectively) and 80 TVT MM were registered in ReNaM (table 1). The mean age at diagnosis was 61.8 [standard error (SE) 2.4] years and 61.4 (SE 3.1) in men and women, respectively, for pericardial MM and 66.7 (1.8) for TVT MM. The gender ratio for pericardial cases (male versus female) was 1.95, ranging from 1.5 in 1993–2003 to 2.6 in 2004–2015. The majority of cases were born before year 1950 in both sites and genders. Histological confirmation was obtained for 87.7% of cases; for 17 cases, only cytology or positive imaging were available. The epithelioid form is predominant in both anatomical sites (37.9% in pericardial cases and 52.5% in testicular cases). Incidence analyses, reported in supplementary table S2, confirm the extreme rarity of these diseases. Mean annual standardized incidence rates for pericardial MM in the overall period 1993–2015 were 0.080 (× 1 000 000 inhabitants) in men and 0.036 in women, using the European standard population as reference (0.049 and 0.023 if world population was used). Pericardial MM incidence rates were higher in 1998–2003 for both genders (0.067 and 0.039 in men and women, respectively, with world population standardization), but no reliable temporal trend analysis was possible. TVT MM mean annual incidence rates were 0.095, with the peak in the same period (0.116 in 1998–2003).

Table 1. Pericardial and tunica vaginalis testis mesothelioma cases by gender, age at diagnosis, periodof incidence, diagnostic certainty, morphology and asbestos exposure. Italian national mesothelioma registry (ReNaM), 1993–2015. [MM=malignant mesothelioma; NOS=not otherwise specified.]

	Pericardial MM				Tunica vaginalis testis MM	
	Women	%	Men	%	N	%
Age classes (years)						
0–44	2	10.0	6	15.8	10	12.5
45–64	8	40.0	9	23.7	15	18.8
65–74	7	35.0	15	39.5	25	31.3
≥75	3	15.0	8	21.2	30	37.5
Period of diagnosis						
1993–1997	3	15.0	5	13.2	8	10.0
1998–2003	9	45.0	12	31.6	23	28.8
2004–2009	2	10.0	11	28.9	24	30.0
2010–2015	6	30.0	10	26.3	25	31.3
Year of birth						
1914–1930	5	25.0	8	21.0	26	32.5
1930–1939	6	39.0	11	29.0	23	28.7
1940–1949	3	15.0	7	18.4	10	12.5
1950–1959	1	5.0	6	15.8	11	13.8
1960–1992	5	25.0	6	15.8	10	12.5
Diagnostic certainty						
MM definite	15	75.0	30	78.9	76	95.0
MM probable or possible	5	25.0	8	21.1	4	5.0
Morphology						
Epithelioid	8	40.0	13	34.2	42	52.5
Biphasic	4	20.0	5	13.2	12	15.0
Sarcomatoid	2	10.0	4	10.5	5	6.3
MM NOS	3	15.0	14	36.8	21	26.3
Not available	3	15.0	2	5.3	-	-
Follow up						
Death	20	95.0	36	94.7	47	58.8
Live at follow up	-	-	2	5.3	33	41.3
Exposure detection						
Indirect interview	14	70.0	20	52.6	23	28.8
Direct interview	3	15.0	8	21.1	45	56.3
No exposure assessment	3	15.0	10	26.3	12	15.0
Modalities of asbestos exposure (only for cases with exposure assessment)						
Occupational (definite)	-	-	8	28.6	25	36.8
Occupational (probable)	1	5.9	4	14.3	5	7.4
Occupational (possible)	3	17.6	9	32.1	15	22.1
Environmental	1	5.9	-	-	1	1.5
Leisure related	-	-	1	3.6	1	1.5
Unlikely	12	70.6	6	21.4	21	30.9
Overall	20	100	38	100	80	100

Table 2a. Cox proportional hazards regression for prognostic factors in survival. Relative risk (RR) and 95% confidence interval (CI) by gender, age at diagnosis, period of incidence, diagnostic certainty and morphology. Pericardial malignant mesothelioma (MM), Italy, 1993–2015 (N=58). [NOS=not otherwise specified.]

	RR	95% CI	P-value
Gender			
Men	1	-	
Women	0.53	0.27-1.02	0.06
Age at diagnosis (years)			
0–64	1	-	
65–74	1.28	0.66-2.49	0.46
≥75	3.52	1.45-8.51	<0.05
Period of incidence			
1993–2003	1	-	
2004–2015	0.76	0.42-1.39	0.38
Diagnostic certainty			
MM definite	1	-	
MM probable or possible	1.50	0.68-3.31	0.32
Morphology			
Epithelioid	1	-	
Biphasic	0.98	0.43-2.24	0.96
Sarcomatoid	1.42	0.53-3.80	0.49
MM NOS	0.74	0.35-1.59	0.44
Not available	1.09	0.36-3.30	0.87

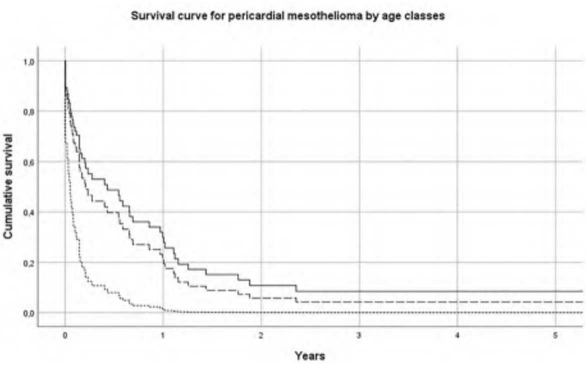

Figure 1a. Survival curve by age-class for pericardial mesothelioma. Italy, 1993–2015 (N=58). [Age classes: ≤64 years = solid line; 65–74 years = long dashed line; ≥75 years = short dashed line.]

The median survival of pericardial MM was 2.5 (SE 1.0) months; 6.8 (SE 0.6) for females and 1.4 (SE 0.6) for males. For TVT MM cases, the median survival was 33.0 (SE 7.8) months. Results of Cox's proportional hazard model for pericardial MM survival showed that older subjects (>75 years) had a hazard risk equal to 3.52 (95% CI 1.45–8.51), with respect to subjects <65 years (table 2a and figure 1a). Pericardial mesothelioma cases with sarcomatoid morphology presented a risk of 1.42 (95% CI 0.53–3.80, P-value 0.6), but this finding, although established in pleural and peritoneal mesothelioma, has to be considered with extreme caution due to the small sample size. Similar findings have been obtained for TVT MM, with a more favorable prognosis for younger patients, (table 2b and figure 1b). Asbestos exposure has been assessed for 113 out of 138 (81.9%) MM cases included in this study, after a direct (56/113=49.6%) or indirect (to a next of kin) interview (57/113=50.5%) (table 1). Occupational exposure to asbestos (definite, probable or possible) was present for 61.9% of interviewed subjects (70/113), with differences by gender and site (23.6% in females pericardial MM, 75.0% in males pericardial MM and 66.2% in TVT MM). The economic sectors more frequently associated with asbestos exposure were construction, steel mills, metal-working industry, textile industry and agriculture. Asbestos exposure in this last sector in Italy has been previously described in ReNaM reports, due mainly to the maintenance of rural buildings containing asbestos.

Table 2b. Cox proportional hazards regression for prognostic factors in survival. Relative risk (RR) and 95% confidence interval (CI) by age at diagnosis, period of incidence and morphology. Tunica vaginalis testis malignant mesothelioma (MM), Italy, 1993–2015 (N=80). [NOS=not otherwise specified.]

	RR	95% CI	P-value
Age at diagnosis (years)			
0–64	1		
65–74	3.31	1.40–7.84	<0.05
≥75	4.93	2.26–10.80	<0.05
Period of incidence			
1993–2003	1		
2004–2015	1.00	0.55–1.82	0.99
Diagnostic certainty			
MM definite	1		
MM probable or possible	1.23	0.39–3.84	0.73
Morphology			
Epithelioid	1		
Biphasic	0.92	0.43–1.97	0.83
Sarcomatoid	0.34	0.08–1.42	0.14
MM NOS	0.99	0.49–1.99	0.97

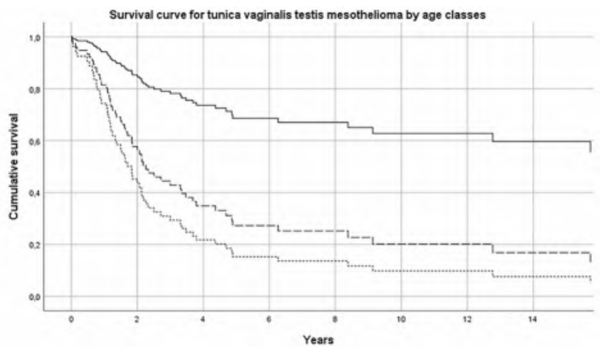

Figure 1b. Survival curve by age-class for tunica vaginalis testis mesothelioma. Italy, 1993–2015 (N=80). [Age classes: ≤64 years = solid line; 65–74 years = long dashed line; ≥75 years = short dashed line.]

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) of pericardial and tunica vaginalis testis mesothelioma by asbestos exposure, from conditional logistic regression models (risk set: age category: adjusted for gender), Italian national mesothelioma registry (ReNaM), 1993-2015. [NC=not calculated.]

Asbestos exposure	Cases	Controls	OR	95% CI
Pericardium MM (women)	17	336		
Occupational	4	37	1.99	0.60-6.63
Occupational (definite/probable)	1	16	1.23	0.15-10.3
Occupational (possible)	3	21	2.55	0.65-10.0
Non-occupational	1	101	0.18	0.02-1.41
Familial	0	46	NC	
Environmental	1	39	0.50	0.06-4.08
Leisure related	0	16	NC	
Unlikely	12	198	1.00	Reference
Pericardium MM, men	28	593		
Occupational	21	208	5.52	2.14-14.2
Occupational (definite/probable)	12	125	5.83	2.06-16.5
Occupational (possible)	9	83	5.45	1.86-16.0
Non-occupational	1	102	0.47	0.06-3.93
Familial	0	42	NC	
Environmental	0	46	NC	
Leisure related	1	14	5.33	0.58-49.4
Unlikely	6	283	1.00	Reference
Pericardium MM, women and men	45	929		
Occupational	25	245	3.68	1.85-7.31
Occupational (definite/probable)	13	141	3.50	1.56-7.84
Occupational (possible)	12	104	3.90	1.76-8.66
Non-occupational	2	203	0.28	0.06-1.21
Familial	0	88	NC	
Environmental	1	85	0.36	0.05-2.77
Leisure related	1	30	1.01	0.13-7.95
Unlikely	18	481	1.00	Reference
Tunica vaginalis testis MM	68	593		
Occupational	45	208	3.42	1.93-6.04
Occupational (definite/probable)	30	125	4.19	2.22-7.90
Occupational (possible)	15	83	2.57	1.25-5.31
Non-occupational	2	102	0.27	0.06-1.18
Familial	1	42	0.31	0.04-2.38
Environmental	0	46	NC	
Leisure related	1	14	1.35	0.16-11.3
Unlikely	21	283	1.00	Reference

Case–control study

We included 45 cases (28 men, 17 women) of pericardial MM and 68 cases of TVT MM. There were 929 controls (593 men, 336 women). There were 74 subjects exposed to asbestos (27 pericardial MM and 47 TVT MM) and occupational exposure was largely predominant (70 MM cases). Considering pericardial MM, an overall significant risk was found for occupational asbestos exposure (OR 3.68, 95% CI 1.85–7.31), with relevant gender difference (OR 5.52, 95% CI 2.14–14.2 and OR 1.99, 95% CI 0.60–6.63 in men and women, respectively) [table 3]. The low number of non-occupationally exposed cases (two cases) leads to statistically unstable risk estimates. The OR of occupational exposure to asbestos for TVT MM risk was 3.42 (95% CI 1.93–6.04). The overall OR of pericardial MM for asbestos-exposed subjects (either occupational or non-occupational) were 1.79 (95% CI 0.95–3.34) and 2.25 (95% CI 1.30–3.90) for pericardial and TVT MM, respectively (data not shown). Sensitivity analyses gave comparable results (tables S7–7), whereas the analyses by economic sectors did not show specific risks.

Discussion

As a legacy of the massive use of asbestos until the 1992 ban, Italy is today one of the countries most affected by asbestos-related diseases. Thanks to a long-term epidemiological surveillance of MM incidence, which covers the Italian population almost completely, our study provides – to our knowledge for the first time – a comprehensive nationwide picture of pericardial and TVT MM epidemiology. Furthermore, this is the first analytical epidemiological study to evaluate the risk related to asbestos exposure for these rare diseases, showing a significant association between occupational

asbestos exposure and both pericardial and TVT MM incidence, supporting the evidence that asbestos cause MM in all anatomical sites.

Pericardial and TVT MM are such extremely rare diseases that incident rates are seldom estimated at the population level. Such a low incidence level prevents exercises of correlation between past asbestos consumption, as a proxy of exposure, and incidence, similar to those performed for pleural and pericardial mesothelioma (25, 26). In asbestos-exposed cohorts, disease rarity prevents any meaningful analysis. Even at the national level, it is very difficult to discuss the epidemiology of pericardial and TVT MM. In Italy, previous cases reports by specialized cancer registries have been published for pericardial and TVT MM at regional (11, 27, 28) and national level (29).

Potential risk factors for pericardial MM, other than asbestos exposure, have been suggested in some case reports or literature reviews: therapeutic ionizing radiation exposure, smoking, chemotherapeutic treatment and history of cardiovascular diseases (11, 16, 30, 31), but no epidemiological study has ever tested these hypotheses. Hydrocele, inguinal hernia or infection and trauma, ionizing radiation and tobacco smoking have been supposed as potential risk factors for TVT MM without experimental or epidemiological confirmations (16, 32). Most of the studies do not contain any information about asbestos exposure. A review of 27 case reports on pericardial MM showned that a third of the patients were exposed to asbestos (33); Guney and colleagues (34) reviewed 74 cases of TVT MM and found that 34.2% of patients presented a history of asbestos exposure. Recently, the need to include the assessment of ionizing radiation or radiotherapy for MM cases registered by ReNaM has been discussed according to the putative role in mesothelioma risk (35).

The main strength of this study is the presence in Italy of a systematic active search of MM over the whole national territory, with standard criteria for case identification, diagnosis classification and evaluation of the occupational, environmental and familial history of affected people, obtained by the means of a structured individual questionnaire. The temporal and territorial extent of the Italian surveillance system comprises >1000 million of person-years of observations. Mesothelioma epidemiological surveillance systems, comparable to Italian experience for information completeness, exposure assessment and territorial coverage, are rare and – to the best of our knowledge – currently present only in Australia, France and South Korea (36–39). Notwithstanding the old age at diagnosis and the large period of recruitment for pericardial and TVT MM cases, the majority of diagnoses were confirmed by histology (78% and 95% respectively). The completeness and quality of diagnosis in ReNaM have been confirmed by comparison with the Italian cancer registries (40). The interview rate was 85% for TVT MM and 78% for pericardial MM, even despite the poor prognosis. All cases and controls included in case–control study have been interviewed using the ReNaM questionnaire, occupational histories were coded with the same classifications of industry and occupation, and exposure was assessed according to the same protocol.

Some limitations of this study have to be considered. Cases of pericardial and TVT MM used in this study have been extracted by ReNaM archives. ReNaM methods in detecting, classifying and coding MM cases have been repeatedly published in the literature (20, 41). ReNaM is collecting MM cases across Italy, but the activity of its regional operating centers did not begin at the same time and this could have biased our study given the inhomogeneous territorial distribution of industrial and natural sources of asbestos exposure. Exposure assessment was qualitative, and the ability to identify the modalities of asbestos exposure effectively was not fully consistent among regional registries despite the use of a shared structured questionnaire. The percentage of collected exposure histories varied between 45–95% among regions. Furthermore, the possible lack of homogeneity among COR in classifying and coding diagnoses and exposures (according to the national guidelines) has to be considered. Finally, as generally is the case with specialized registries, potential over-reporting could be a concern for ReNaM. Limitations of the case–control study is that control samples refer to previous studies conducted between 2012 and 2015 with a temporal mismatch with MM cases (1993–2015). The incomplete time coverage of controls was partially compensated by being age-matched and the fact that we considered lifetime asbestos exposure, which is likely to be fairly constant after the 1992 asbestos ban in Italy, even if the amount of asbestos-containing materials removed after the ban was consistent and could introduce a bias that is difficult to assess. Furthermore, lifetime occupational asbestos exposure among male cases and controls showed little variation except for those born on or after 1960. Sensitivity analyses with temporal restrictions (only period 2000–2015 and only subjects born before 1950) were in line with the results of the main analysis (tables S5 and S6). Geographical coverage of controls was also incomplete, with controls enrolled only from Apulia, Lombardy, Piedmont, Tuscany, Veneto and Emilia-Romagna regions. These regions were (and still are) among the most industrialized areas in Italy, and it is highly plausible that lifetime asbestos exposure is higher in the population living in these regions than in other regions that did not provide controls. Case–control sensitivity analyses, restricted to the six regions which enrolled control subjects, yielded ORs that were comparable with those found in the main analysis. Finally, the

qualitative reconstruction of exposure and the contemporary use of chrysotile and amphiboles in many occupational settings in Italy did not allow for any separate analysis between different varieties of asbestos fibers.

In conclusion, this study provided further evidence of the extreme rarity of pericardial and TVT MM, with a mean annual incidence rates in Italy <1 case per 10 million person-years. Survival analyses confirmed the very poor prognosis for pericardial MM and the prognostic role of age for both pericardial and TVT MM (more favorable for younger patients). Finally, for the first time, our analytical epidemiological study showed an association between asbestos exposure and pericardial and TVT MM risk, supporting the causal role of asbestos for MM of all anatomical sites.

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Competing interests

Dario Consonni, Carolina Mensi, Dario Mirabelli and Corrado Magnani served as consultants for the court in trials concerning asbestos-related diseases.

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Ethics approval

As reporting of malignant mesothelioma to the National Mesothelioma Registry (ReNaM) is compulsory by law (277/1991 and 81/2008), ethics approval is not required for cases. The MISEM and CARA studies (used for control analyses) were approved by the following institutional review boards: Comitato Etico Interaziendale, AOU San Giovanni Battista di Torino and AO CTO/Maria Adelaide, Torino, Italy and Comitato Etico del Policlinico di Sant'Orsola, Bologna, Italy

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